THE UNIVERSITY OF ALBERTA

NUCLEAR BEHAVIOR AND NUCLEAR STRUCTURE OF THE FUNGUS CYATHUS

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

DATE Sept. 18, 1962

SYNOPSIS

Two species of the fungus Cyathus (Nidulariaceae) were investigated cytologically with respect to nuclear behavior. Cyathus stercoreus was the main object of investigation. In this species chromosome cycles (meiosis and mitosis) and chromosome morphology are described, and their evolutionary implications from a cytogenetic view point are discussed. The fusion of two nuclei of compatible mating type takes place in the developing basidium at the end of telophase of the presynaptic mitosis. Synapsis follows immediately after nuclear fusion. During synapsis the chromosomes elongate, facilitating point-to-point pairing. Meiosis as well as mitosis are essentially similar to those processes in higher organisms. Details of divisional stages are described and illustrated with photomicrographs. The presence of centrioles and spindles is demonstrated. A paracentric inversion was found on the long arm of chromosome 3. Also a double chromatid bridge without acentric fragments was found in telophase I. Karyotype analysis reveals that, of 12 chromosomes, there are two sub-sets of 6; within each sub-set, chromosomes exist in pairs regarding size and form. This evidence, together with the presence of quadrivalents as well as secondary associations of like chromosomes suggests that C. stercoreus may be an autotetraploid species. The suggestion

that tetraploidy might be a mechanism for the natural evolution of the tetrapolarity of sex factors is brought forward.

ACKNOWLEDGEMENT

The writer is indebted to Dr. Harold J. Brodie for his encouragement and his constant guidance; his criticism of the manuscript is also acknowledged. This work is made possible by a grant to Dr. Brodie and a studentship to the writer from the National Research Council of Canada, Ottawa, Canada.

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INTRODUCTION

That the knowledge of chromosome morphology and chromosome behavior has been a great asset to the study of higher plants has been shown by the recent advances in cytogenetics and cytotaxonomy. The techniques of the latter disciplines have been very little used by mycologists, however, partly because of the difficulty of revealing chromosome morphology and chromosome behavior among the fungi.

Since Neurospora became well known as a tool for genetic studies, basic cytological studies have been made of several fungi. Outstanding among these are the work of McClintock (29) and Singleton (39) on Neurospora crassa, that of Carr and Olive (16) on Sordaria fimicola, and that of Knox-Davies and Dickson (24) on Helminthosporium turcicum. In the Basidiomycetes, comparable definitive demonstrations of nuclear behavior and chromosome structure are scarce although genetically a few fungi of this group have been studied extensively (33).

Lu and Brodie (27), in a recent paper, reported that the chromosomes of the fungus Cyathus are relatively large. This suggests that Cyathus may be good material for a detailed investigation of fungus chromosomes. Cyathus, a member of the Gasteromycetes, has been studied by Brodie (2, 5, 6) regarding growth characteristics in culture and regarding the structure of fruit bodies; by Brodie (3) and Fulton (21) regarding sexuality and diploidization; and by Garnett (22) and Lu (25) regarding fruiting behavior. The latter author has investigated some of

the conditions necessary for the induction of fruiting. The results suggest that it may be possible to obtain fruit bodies with regularity under laboratory conditions, an accomplishment that would greatly facilitate further research, especially of a genetic nature.

If Cyathus is to be used for cytogenetic and cytotaxonomic investigations and is to be studied phylogenetically, basic cytological information is indispensable. For this reason the investigations reported in this paper were undertaken, the chief objectives being to follow the chromosome cycle (both meiosis and mitosis) and to study and record chromosome morphology.

The genus Cyathus is one of the best known and most widely distributed fungi of the family Nidulariaceae (Bird's Nest Fungi). Detailed accounts of the life cycle and development of several species have been given by Brodie (2, 3, 4, 5, 6). Cyathus stercoreus (Schw.) de Toni is a predominantly coprophilous species of common occurrence in North America; C. olla Pers. is equally common, but is found mostly on garden soil containing compost.

In all species of Cyathus, as far as is known, basidiospores are haploid and usually binucleate. Basidiospores, upon germination, produce haploid or monokaryotic mycelia. When they unite in sexually compatible pairs, haploid mycelia give rise to diploid or dikaryotic mycelia characterized morphologically by the presence of clamp connections and the ability to fruit. Sexuality is universally of the tetrapolar

or four-mating-type pattern, the interactions of haploid mycelia being determined by the action of two pairs of factors.

Di karyon mycelia have (with a high degree of regularity) two nuclei in each cell of the mature parts of the mycelium. Growing hyphal tips may occasionally contain an irregular number of nuclei. The two regularly associated nuclei constitute the dikaryon, that is a pair of nuclei which are genetically compatible and which will ultimately unite in the basidium (e. g. of genotypes AB + ab or Ab + aB). The nuclei of the dikaryon divide conjugately as the dikaryotic mycelium grows. The nuclei do not fuse, however, until basidia are finally formed in a young developing fruit body (basidiocarp).

Fruit bodies arise as subterminal knots on special strands of dikaryon mycelium. Each fruit body is early differentiated into a gleba -- a central portion in which sporogenous tissue develops -- and a bounding wall or peridium. Within the gleba, matrical hyphae become organized into highly specialized reproductive units called peridioles (5). The peridiole consists of an outer layer, the tunica; and an inner cortex. The inner portion of the cortex bears a hymenium of basidia, each young basidium containing a pair of non-sister nuclei -- the dikaryon.

The two nuclei comprising the dikaryon fuse ultimately in the basidium, and meiosis follows. One haploid nucleus resulting from meiosis passes into each basidiospore via a sterigma. The haploid

nucleus in each spore usually undergoes one mitotic division as the spore is maturing. Thus basidiospores are haploid and are genetically segregated into the four mating types AB, ab, Ab and aB *.

MATERIALS AND METHODS

Two species of Cyathus were studied, Cyathus stercoreus being the chief object of investigation. In this species, chromosome cycles and chromosome morphology were investigated in detail. Cyathus olla was also studied but mainly to show the similarity, in the behavior of chromosomes, between the two species, and to provide any supplementary information that might be forthcoming.

Mature fruit bodies of C. stercoreus were obtained from Dr. Brodie, whose strain #1305 (from a garden, Edmonton, Alberta, July, 1960) was used for the present studies. Peridioles were removed from the basidiocarps and were sterilized in 0.5% solution of mercuric chloride for 2 minutes. After 3 subsequent washes (2 minutes each) in sterilized distilled water, peridioles were teased into small pieces. The suspensions so obtained were then incubated at room temperature for a week or so. The new growth of the dikaryon mycelium (diploid) from the peridiole-wall tissues was then transferred to, and maintained in, slant cultures.

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*Other symbols have been used by other investigators of sexuality in Basidiomycetes: e.g. ab, a^1b, ab^1, a^1b^1, etc. The symbols used above are preferable for visual simplicity and it need only be noted that the capital letters do not indicate genetic dominance.

Induction of Fruit Bodies

Cultures of the above dikaryon mycelium were prepared on Brodie's agar medium (2) in 14 cm Petri dishes, and were grown at 25°C under a 16-hour-per-day illumination at a light intensity of 275 foot-candles. Fruiting was initiated (appearing as hyphal knots) 16 days after inoculation. Material showing various stages of the chromosome cycle was obtained from the young developing fruit bodies more or less by trial and error. Generally speaking, fruit bodies were ready for fixation and staining after 3-4 days' development, at which time they were 5-7 mm x 10-12 mm in size. The size of fruit bodies suitable for cytological study varied greatly with changes in the environmental conditions (25). Two characteristics of the fruit bodies were noted as indicating the optimum time for fixing: (a) fruit bodies were round and fluffy-headed (as when first formed) and there was as yet no sign of the formation of an epiphragm on the top of the fruit bodies; (b) peridioles were white and soft although they had reached their maximum size. In contrast, a fruit body was found to be too old for chromosome studies if it had a dark circle on the top, indicating the development of an epiphragm, and if the peridioles were brown and hard.

Fixation and Staining

Young developing fruit bodies were harvested (fruit bodies of

C. olla were collected directly from an Edmonton garden) and fixed for 1-3 days in BAC fixative (9 parts butyl alcohol, 6 parts glacial acetic acid and 3 parts 10% aqueous chromic acid) as described by Lu (26). Peridioles, which had been removed from the fixed fruit body, were hydrolyzed in an HCl-alcohol mixture for 12 minutes at 60°C. After being washed in Carnoy's fixative for 5-10 minutes, the peridioles were squashed on a slide and stained with the improved propionocarmine staining technique, which has been published elsewhere (26).

For staining the mycelium, a lump of it (living) was teased into very small pieces in sterilized distilled water in a Petri dish, where the mycelium was allowed to continue growth for 24-36 hours. The actively growing mycelia were then fixed, hydrolyzed and stained.

Special Technique for Spreading

In the Basidiomycetes, the basidia are so small that special means are necessary to spread chromosomes for study. Unless basidia are well spread out as a "smear" it is impossible to obtain a good preparation. In order to achieve the desired spreading all basidia were cut away from the sporogenous tissue, and the debris, such as cortical tissue, was removed as cleanly as possible. The following technique has been used with success.

After hydrolysis and washing, the tunica (the outer cover) of the peridiole was removed, leaving only the inner layer, which is the sporogenous tissue where basidia are borne. The sporogenous tissue was then removed to a clean slide to which 2-3 drops of staining solution were added. Transverse sections of this material were cut with a pair of very fine dissecting knives. The sections were heated gently in the staining solution for a couple of seconds. With a pair of very fine needles, the inner sporogenous tissue of the sections was carefully dissected under a microscope to separate the basidia. After placing the latter under a No. 0 cover slip, they were heated gently, then pressed (cover slip down) against blotting paper. When the soft cytoplasm of the basidium flowed sideways, the chromosomes were spread out. Sometimes, a few taps on the cover slip with a needle before pressing was found to cause further spreading. This tapping also brought nuclei out of the spore (Fig. 52) when otherwise the staining of nuclei might not have been successful.

Microscopy

Direct observations were made and photographs taken with a Zeiss Phase-Contrast Microscope (oil immersion lens N. A. 1. 30). A light blue filter (Walz B. 9) was used. Photographs were taken with a Leitz-Wetzlar camera using Kodak Contrast Process Ortho film, developed with Eastman D-11 developer, and printed on Kodabromide paper.

THE CHROMOSOME CYCLES

1. Meiosis

Presynaptic Nuclei and Synapsis

In a developing peridiole, two nuclei of compatible mating type in certain hyphae of the sporogenous layer undergo presynaptic conjugate (mitotic) division. At late telophase of the latter, each nucleus forms a nuclear membrane. Meanwhile, the ultimate cell of each of such hyphae develops into a basidium, and at the same time, two nuclei approach each other (Figs. 1-4, 54-57) and fuse (Figs. 5-7). Following nuclear fusion, synapsis begins (Figs. 8, 58). Subsequently, the penultimate cell also develops a basidium on one side of the apical end, possibly from the clamp connection (Figs. 55, 59, 60a). Two nuclei may either approach each other and undergo fusion and synapsis in the cell before entering into the neck of the developing basidium (Fig. 59), or they may enter into the neck of the developing basidium prior to nuclear fusion and synapsis (Fig. 60a). During karyogamy, two nuclei are often seen in the neck of the developing basidium (Figs. 3-8, 54-56). These nuclei, generally but not exclusively, approach each other in such a manner that the chromosomes of the two nuclei face one another, leaving the nucleoli farther apart (Figs. 1-3, 54, 57, 58, 60a). This may be because two nuclei of a dikaryotic mycelium are always so arranged (Fig. 79).

PLATE I -- Presynaptic Nuclei and Synapsis (Cyathus stercoreus)

Figs. 1-5, Presynaptic nuclei:

Figs. 1 and 2. Two nuclei approaching one another, the nuclear membrane not yet formed.

Fig. 3. Two approaching nuclei with nuclear membrane, the nuclei in neck of basidium.

Figs. 4 and 5. Nuclear fusion taking place at point of contact where nuclear membrane dissolves; nuclei in neck of basidium.

Figs. 6-8, Synapsis:

Fig. 6. Two nuclei fusing. Thread-like chromatin and two centrioles are discernible and nucleoli are present.

Fig. 7. Nuclear membrane increased in size. Within the membrane two nuclei are closely appressed.

Fig. 8. Synapsis begins. Arrows point to where pairing of chromosomes (contributed by two nuclei) is taking place from the tip of the chromosomes.

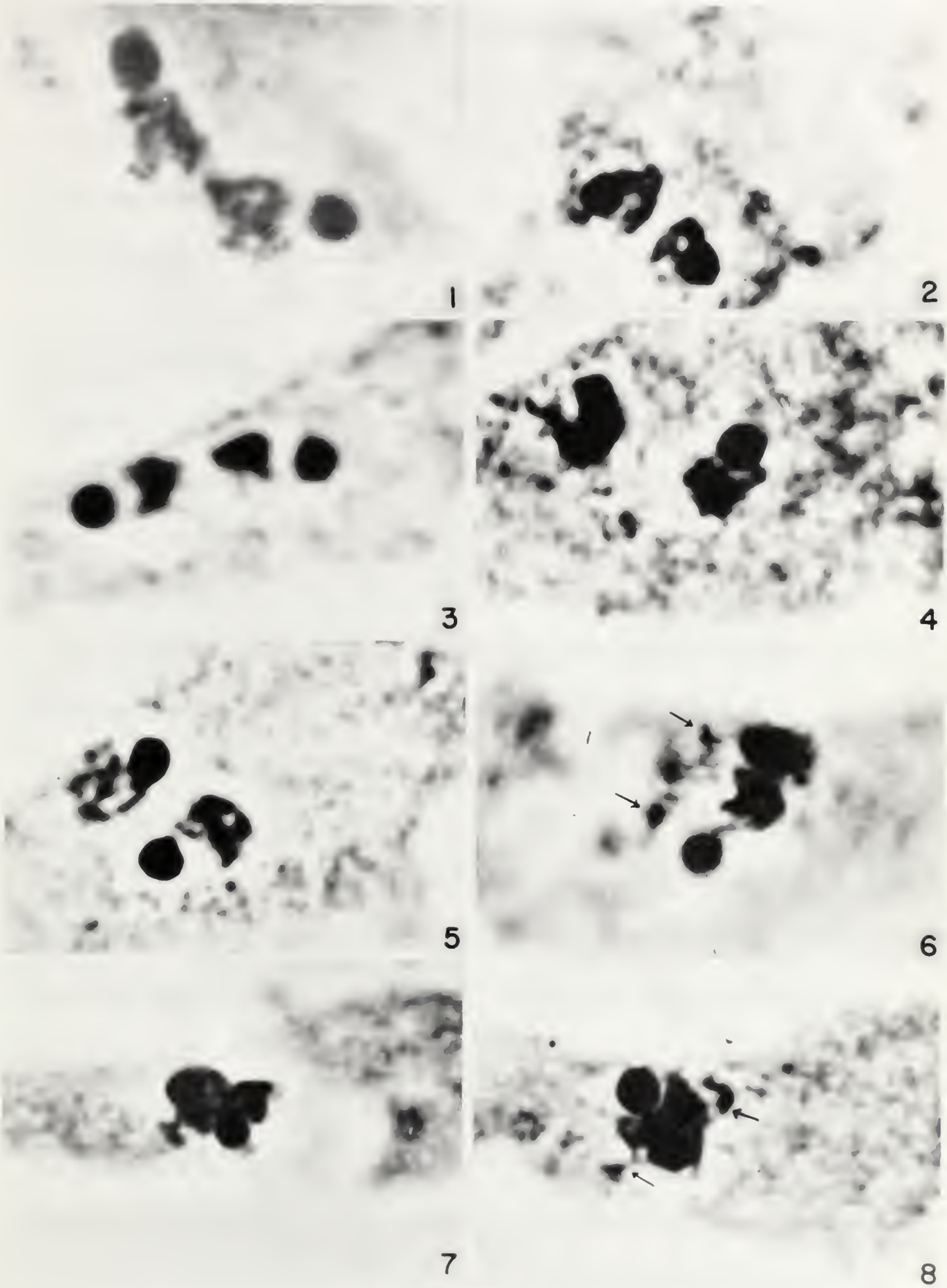


PLATE 1

The process of uncoiling facilitates synapsis (Fig. 58). It is conceivable that, in order to have perfect pairing of homologous chromosomes, complete uncoiling is necessary. The synapsis is believed to have taken place from the point of first contact and then to have proceeded throughout the length of the chromosome. In Fig. 8 are shown two chromosome pairs in which synapsis has started from the end. The fusion of nucleoli cannot be followed exactly. Nevertheless, nucleoli would appear to be neither the first nor the last to fuse, because when synapsis has begun, nucleoli have not yet fused (Figs. 7-8, 58), whereas, when the nucleoli have fused, the synapsis is not yet complete (Fig. 61). Probably the fusion of the nucleoli takes place when the nucleolar chromosomes complete their pairing. At the completion of synapsis or thereabouts, the diploid nucleus is located in the main body of the basidium (Fig. 60b).

The First Meiotic Division

Pachytene: At the completion of synapsis, where chromosomes have stretched to the utmost (Figs. 9, 62), the thread-like chromosomes now undergo their current coiling. Meanwhile, they become duplicated to form a four-strand stage. As the coiling proceeds, chromosomes become shorter and thicker (Figs. 10-19, 63-66) perhaps due to the fact that the basic (minor) coiling of chromosomes has spiralized into the major coiling. At this time chromosomes are very distinct and their individual morphology may be observed (Figs. 17-19, 53).

PLATE II -- Progressive Stages of Pachytene after Synapsis

(Cyathus stercoreus)

Fig. 9. Early pachytene, immediately after synapsis. Very fine thread-like chromosomes entangled; nucleolus present, connected to chromosome 12.

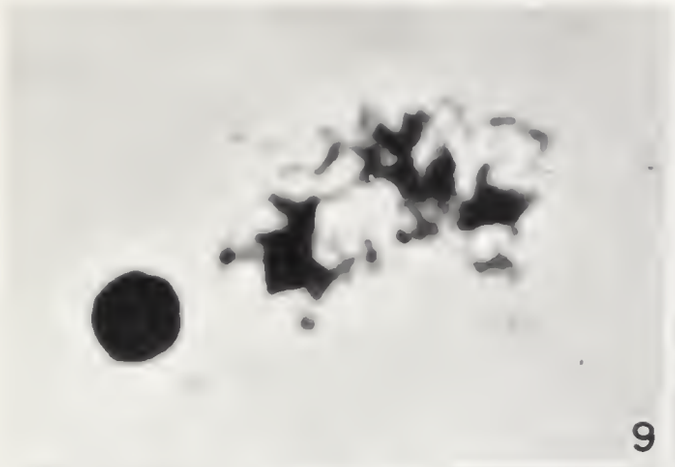
Figs. 10 and 11. Early pachytene, chromosomes shorter and thicker. Note in Fig. 11, the satellite of chromosome 12 sticks out from the nucleolus.

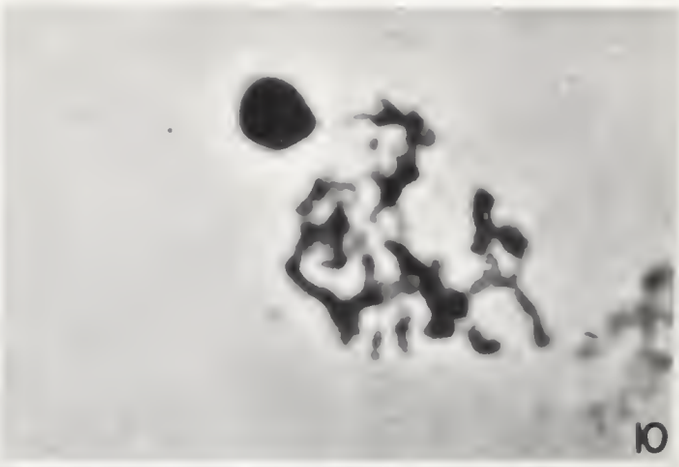
Fig. 12. Pachytene, showing the loop of chromosome 3 (arrow); note chromosome 12 and its satellite.

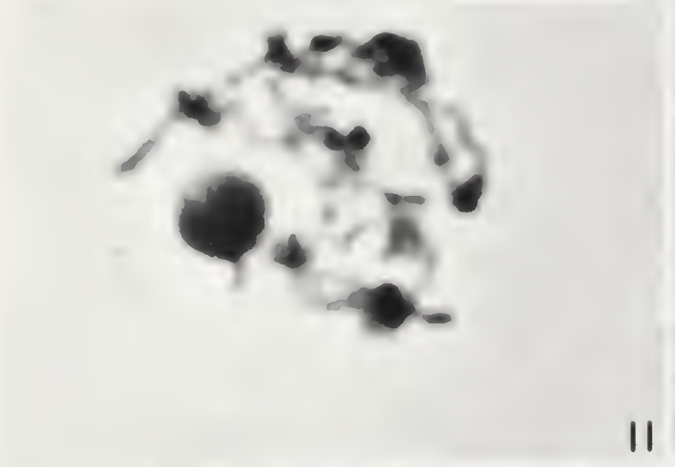
Fig. 13. Pachytene, arrow points to the long arm of chromosome 1 where a heterochromatin knob is seen on 2/5 of the arm toward the tip.

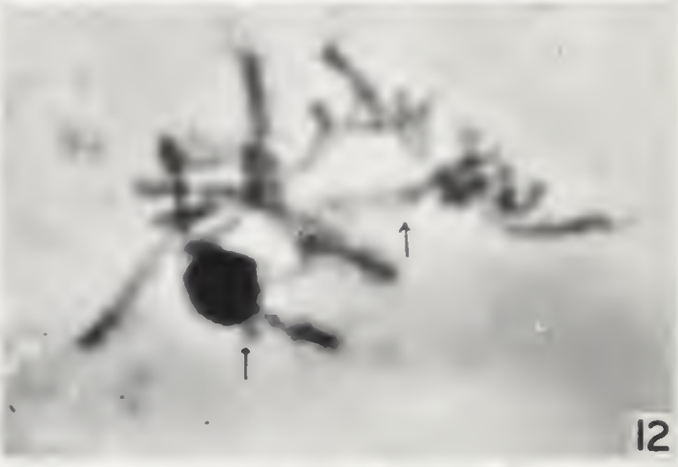
Fig. 14. Pachytene, showing the satellite of contracted chromosome 12; chromosome 1 near the nucleolus.

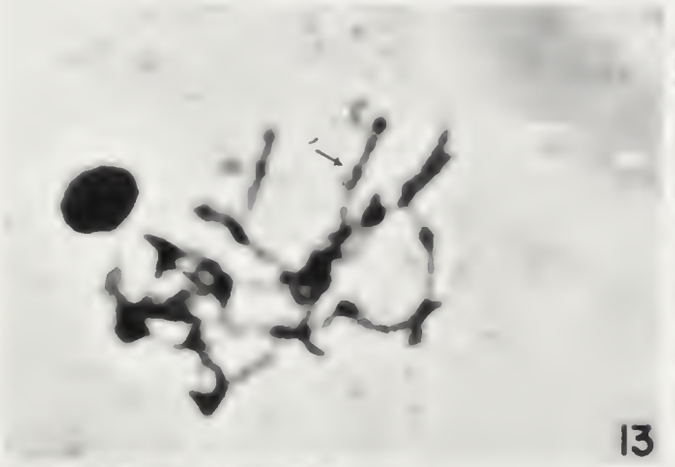
Figs. 15 and 16. Pachytene, showing entangled chromosome in the center of the basidium. Note the curved chromosome 11 in Fig. 15 (arrow).

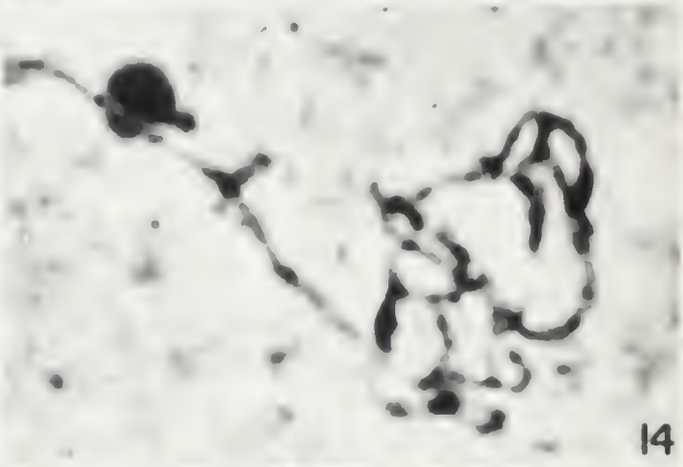












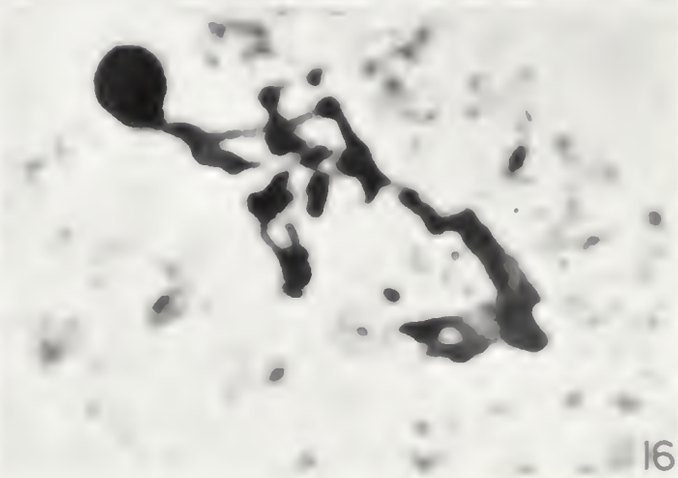


PLATE 11

Plate III (continued)

Fig. 22. Diplotene, arrow points to probably centromere position of chromosome 11.

Figs. 23-24. Diplotene, arrow points to chromosome 3 the loop of which has contracted to a large knob. Chromosome 4 which has a large telomere on its long arm, showing chiasma on the long arm. Note the satellite and the secondary constriction of the chromosome 6. Chromosomes 7 and 8, 9 and 10, as well as 11 and 12 form quadrivalents. Chromosomes 1 and 2 constitute the central line.

PLATE III -- Late Pachytene and Diplotene for Chromosome Morphology (Cyathus stercoreus)

Figs. 17 and 18. Late pachytene, showing individual chromosomes except chromosome 10 (not in focus) near chromosome 9. Arrow points to the loop of chromosome 3. Centromere position of chromosomes 1, 6 and 7 may be determined by chromosome shape. Note the groupings of like chromosomes.

Fig. 19. Late pachytene (see Fig. 53 for individual chromosomes), centromere position for chromosomes 1, 2, 4, 5, 6, 7, 8 and 12 can be determined. The long arm of chromosome 1 bends to one side indicating that there is a heterochromatic knob (compared with Fig. 14) following a secondary constriction. Arrow (above) points to the loop of chromosome 3; arrow (below) points to chromosome 12 at its satellite. The satellite of chromosome 6 bends over because of a secondary constriction (compare with Fig. 17). Note the groupings of like chromosomes.

Fig. 20. Early diplotene, loops beginning to form as seen at the short arm of chromosome 1; centromere position of chromosomes 1, 2, and 4 can be determined. Note the satellite of chromosome 6 (arrow pointed).

Fig. 21. Early diplotene, arrow points to chromosome 6 which shows its centromere next to a heterochromatic knob on the long arm.

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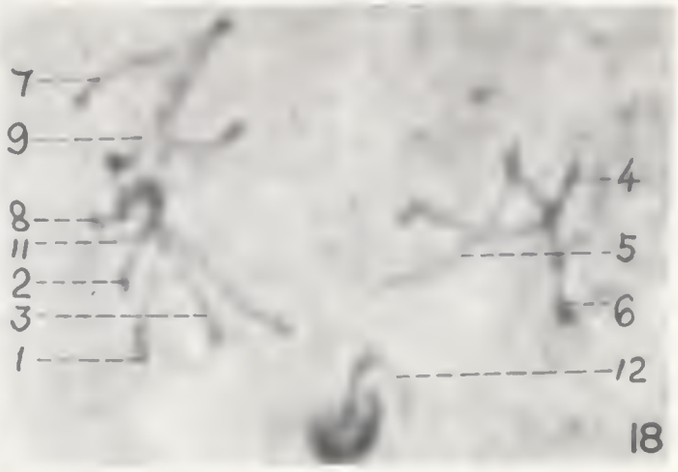

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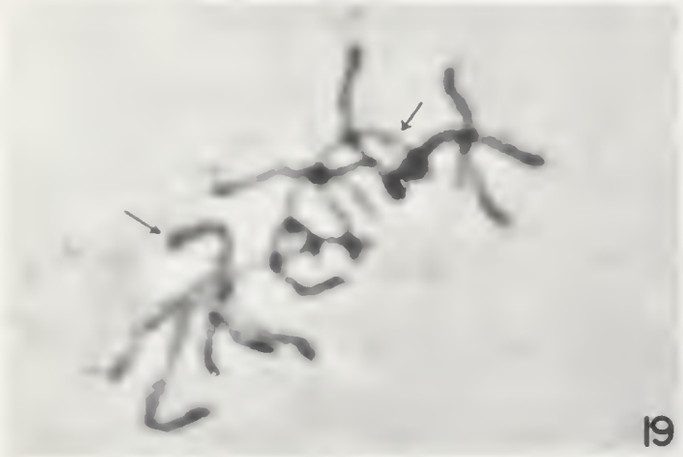

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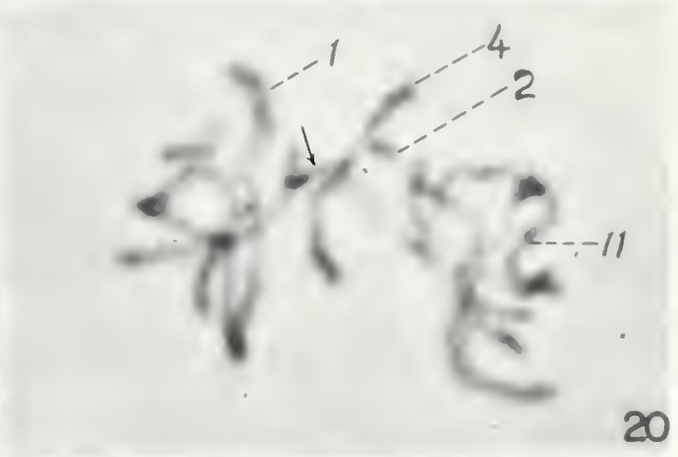

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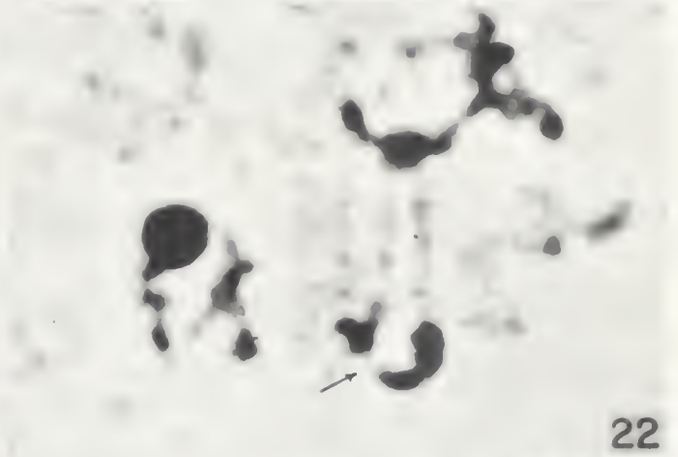

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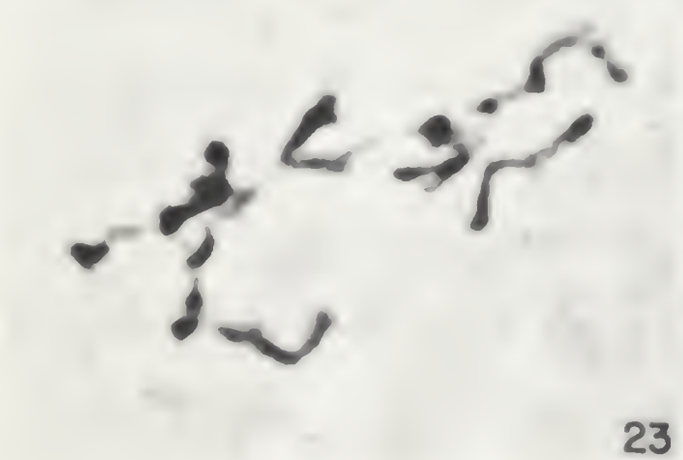

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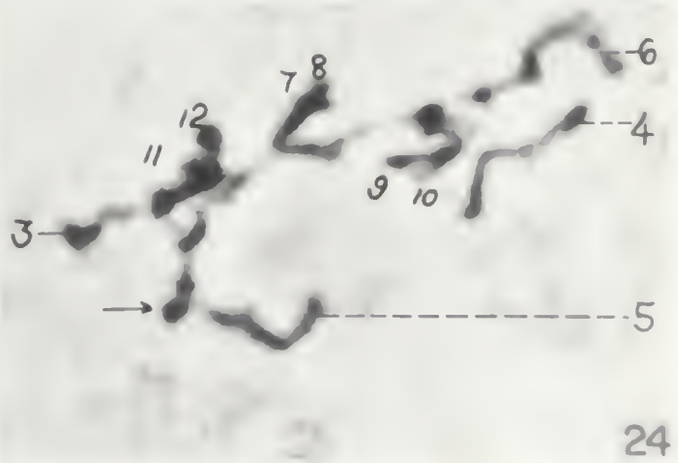

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PLATE III

The pachytene stage occupies a considerable span of time, being probably the longest stage in the whole meiotic cycle. It has been observed that chromosomes at this stage vary in length considerably from fairly long to rather short. They range in length from 13 μ for the longest (chromosome 1) to 3 μ for the shortest (chromosome 12) at the stage shown in Fig. 19. "Bivalentness" has been very clearly demonstrated in chromosomes of C. olla (Figs. 64-66). Since the major coiling occurs only within each homologous pair of the bivalent chromosomes (41), further contraction then brings about some tension, which in turn contributes to the repulsion between two homologues whereby loops are formed. This development marks the beginning of diplotene (Figs. 20, 67).

Diplotene: At diplotene, chromosomes contract very quickly, perhaps as the result of the spiralization of the major coiling into supercoiling. The homologues of the bivalents separate partially; and loops may be seen in separating homologues (Figs. 21-25, 67-71). Relational coilings may still be seen (Figs. 25, 67), and chiasmata are discernible (Figs. 23-24, 68).

Diakinesis: Diakinesis and diplotene actually represent two aspects of one process, the supercoiling. However, it is difficult to draw a clear line between them. Diakinesis can be characterized

PLATE IV __ Late Diplotene, Diakinesis to Early Metaphase I
(Cyathus stercoreus)

Fig. 25. Late diplotene, arrow (above) points to the secondary associated chromosomes 1 and 2; arrow (below) points to chromosomes 9 and 10 showing quadrivalent pairing. The chromosome at "six o'clock" position shows duality of bivalent.

Fig. 26. Early diakinesis, arrow points to chromosome 2 showing centromere position. Note the large satellite of chromosome 12 at "12 o'clock" position (arrow).

Fig. 27. Diakinesis, showing groupings of like chromosomes; from left to right are chromosomes 5 and 6, chromosomes 1 and 2, Chromosome 1 shows the pulling force at the separated centromeres. Arrow (to the left) points to chromosomes 3 and 4 that form a quadrivalent (a chain of four). Arrow (to the right) points to the satellite of chromosome 12 overlapping with chromosome 5 as well as with chromosome 4.

Fig. 28. Diakinesis: The chromosome at the left shows a quadrivalent pairing (a ring of four). Arrow to the left points to chromosomes 3 and 4 which form a chain of four quadrivalent. The chromosome at the far right also shows quadrivalent pairing (a figure 8). Arrow to the right points to the satellite which is overlapped with chromosome 5 as well as with chromosome 4.

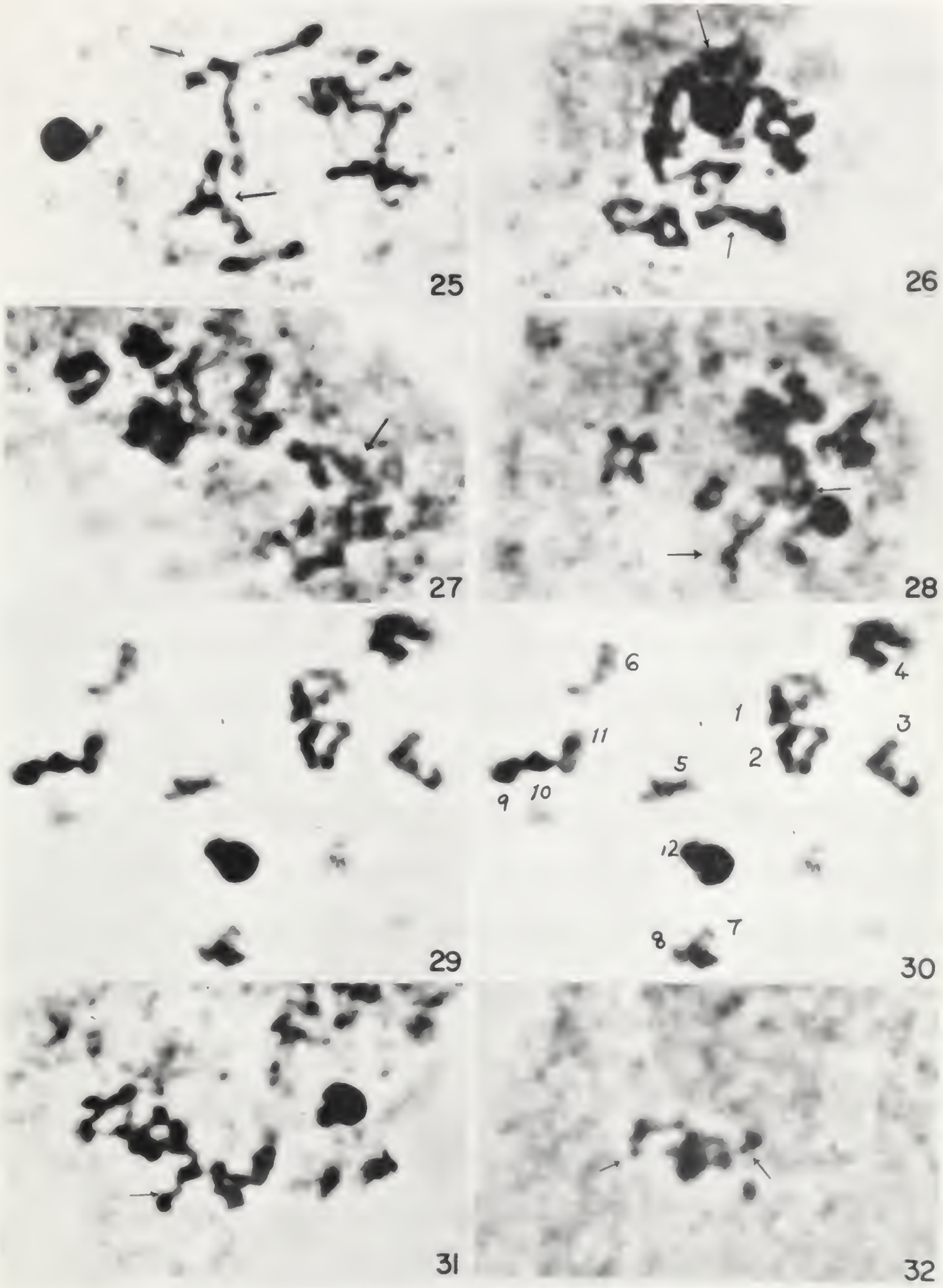


PLATE IV

by the separation of two homologous centromeres, and by the terminalization of chiasmata (Figs. 26-31, 72). Here the bivalents appear either to have the form of a ring (if chiasmata persist) or the form of a chain (if one of the chiasmata opens) (Figs. 26-31, 72).

Metaphase I: Chromosomes continue to condense to the maximum and at this time, they measure from 0.5 to 1 μ in length (Figs. 32, 33). Two centrioles then appear (Fig. 32) and the nucleolus is detached from the nucleolar chromosome and passes into the cytoplasm. Meanwhile, the chromosomes align themselves at the equatorial plate around the spindle (Figs. 34-36, 73) which is formed between the two centrioles. The long axis of the spindle lies normally at approximately right angles to the long axis of the basidium (Figs. 35, 73). Because the chromosomes at this stage are extremely small and they clump together at the equatorial position, resolution of the individual chromosomes is practically impossible (Figs. 32-36, 73). Indeed, the chromosomes could not be completely resolved even in one instance where polar smearing was obtained (Fig. 33).

Anaphase I: Unlike the anaphase of higher organisms, the poleward movement of the dyads of this fungus is not synchronous (Figs. 37-38). Clear evidence for this can be seen in Figs. 37 and 38. In Fig. 37, the first pair of the bivalents has moved towards the

PLATE V -- Metaphase 1 through Early Telophase I
(Cyathus stercoreus)

Fig. 33. Metaphase I, polar view, chromosomes not entirely resolved.

Fig. 34. Metaphase I, showing chromosomes clumped at equatorial plate, two ovoid centrioles at polar positions,, and chromosomal fibres. Within the chromosomal fibres is the continuous spindle which forms between two centrioles.

Fig. 35. Metaphase I, note the long axis of the spindle at right angles to the long axis of the basidium.

Fig. 36. Metaphase I, arrow points to an ovoid body often found associated with one of the centrioles (the nature of this body is not understood).

Fig. 37. Anaphase I, showing non-synchronous chromosome movement. Note the first pair of bivalents which have moved toward the opposite poles while the rest remain at equatorial plate.

Fig. 38. Anaphase I, all chromosomes except 3 bivalents have reached the poles. Note 3 strings of chromosomal fibres corresponding to 3 bivalent pairs. This suggests that chromosomal fibres are definite in number.

Fig. 39. Early telophase I, showing two daughter chromosome groups at polar positions. The nucleolus is freed in the cytoplasm. Note a double chromosome bridge without acentric chromosome fragments.

Fig. 40. Telophase I, chromosomes (the dyads) are uncoiling. Arrow points to the developing nucleolus.

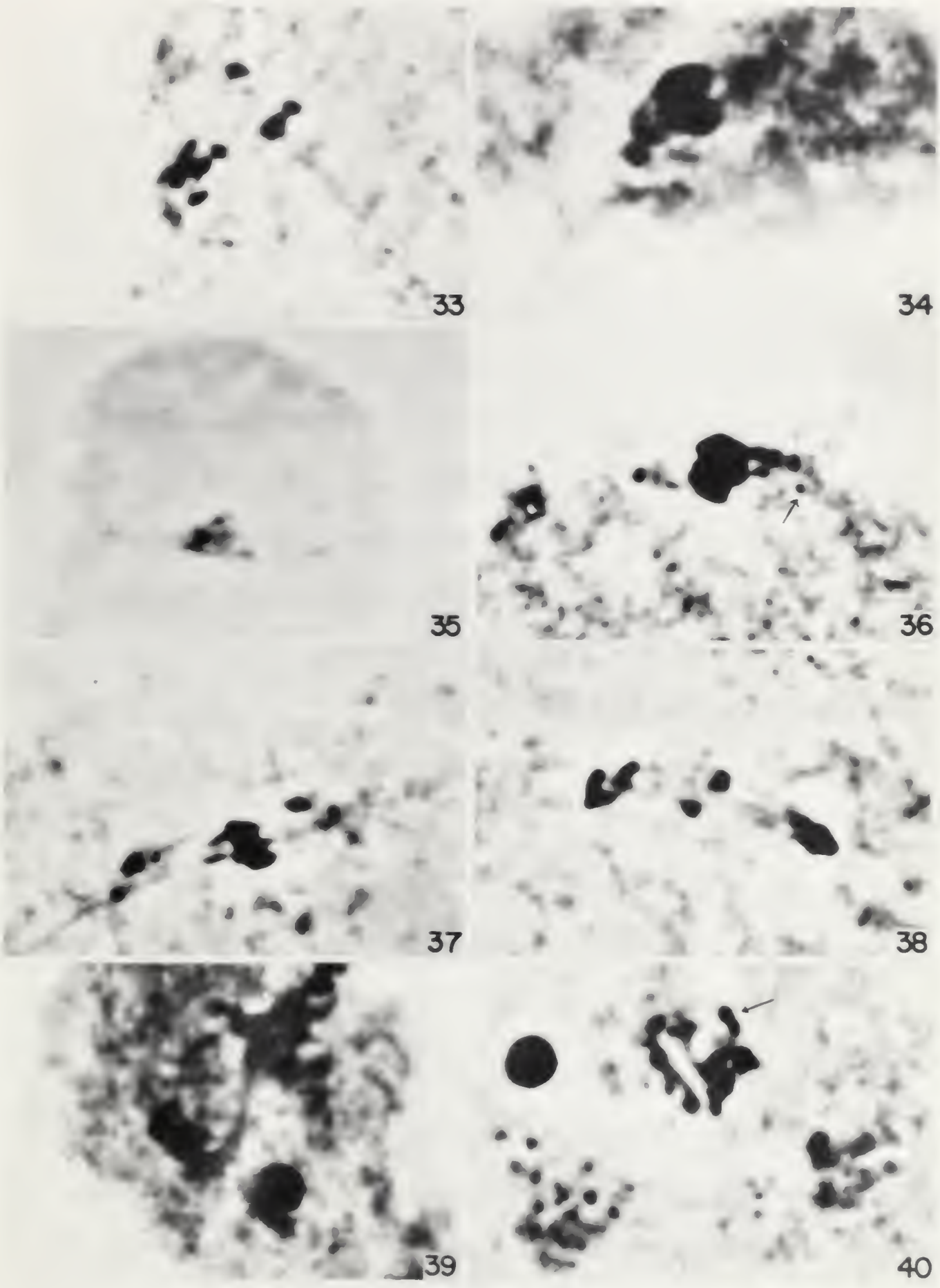


PLATE V

centrioles while the rest of the chromosomes has apparently not moved. On the other hand, in the nucleus shown in Fig. 38, most dyads have arrived at the poles, yet three pairs still remain in the equatorial position.

Telophase I: After all dyads have reached the polar positions, they remain grouped together (Fig. 39). Meanwhile, these dyads begin to loosen or uncoil and the nucleolus is being reorganized by the nucleolus organizer (Figs. 40-41). At this time the centriole is still discernible (Fig. 42). At early telophase, chromosome bridges are observed occasionally (Fig. 39). The nature of these bridges is not fully understood.

Interphase I: Interphase I represents a transitory stage in the process. It is not a resting stage by any means. Chromosomes at this stage have uncoiled to the maximum while remaining attached to the centriole, perhaps by chromosomal fibres (Fig. 43). The centriole has become less stainable.

The Second Meiotic Division

At prophase II, the dyads undergo coiling which makes the chromosomes appear like those of the pachytene stage although thinner (Figs. 44-45). Further contractions bring chromosomes into prometaphase II, where 12 dyads are clearly distinguishable (Fig. 47).

PLATE VI -- Telophase I through Metaphase II
(Cyathus stercoreus)

Figs. 41-43. Telophase I to Interphase I:

Fig. 41. Telophase I, showing uncoiling of chromosomes, nucleolus formed.

Fig. 42. Telophase I (from the same basidium as Fig. 41), showing further uncoiling. Arrow points to the remaining centriole.

Fig. 43. Interphase I, arrow points to the remaining centriole, connected to chromosomes probably with chromosomal fibres.

Figs. 44-48. (magnification 3200 X) Second meiotic division.

Fig. 44. Prophase II (the nucleus to the right), anaphase II (left). Arrow points to a nucleolus disintegrating in the cytoplasm.

Fig. 45. Prophase II (left), anaphase II (at "12 o'clock" position). Arrow points to the nucleolus, free in cytoplasm.

Fig. 46. Prophase II (left), anaphase II (right).

Fig. 47. Prometaphase II (left), the nucleus to the right is not in focus.

Fig. 48. Metaphase II (below) and anaphase II (above). Note the ovoid centrioles.

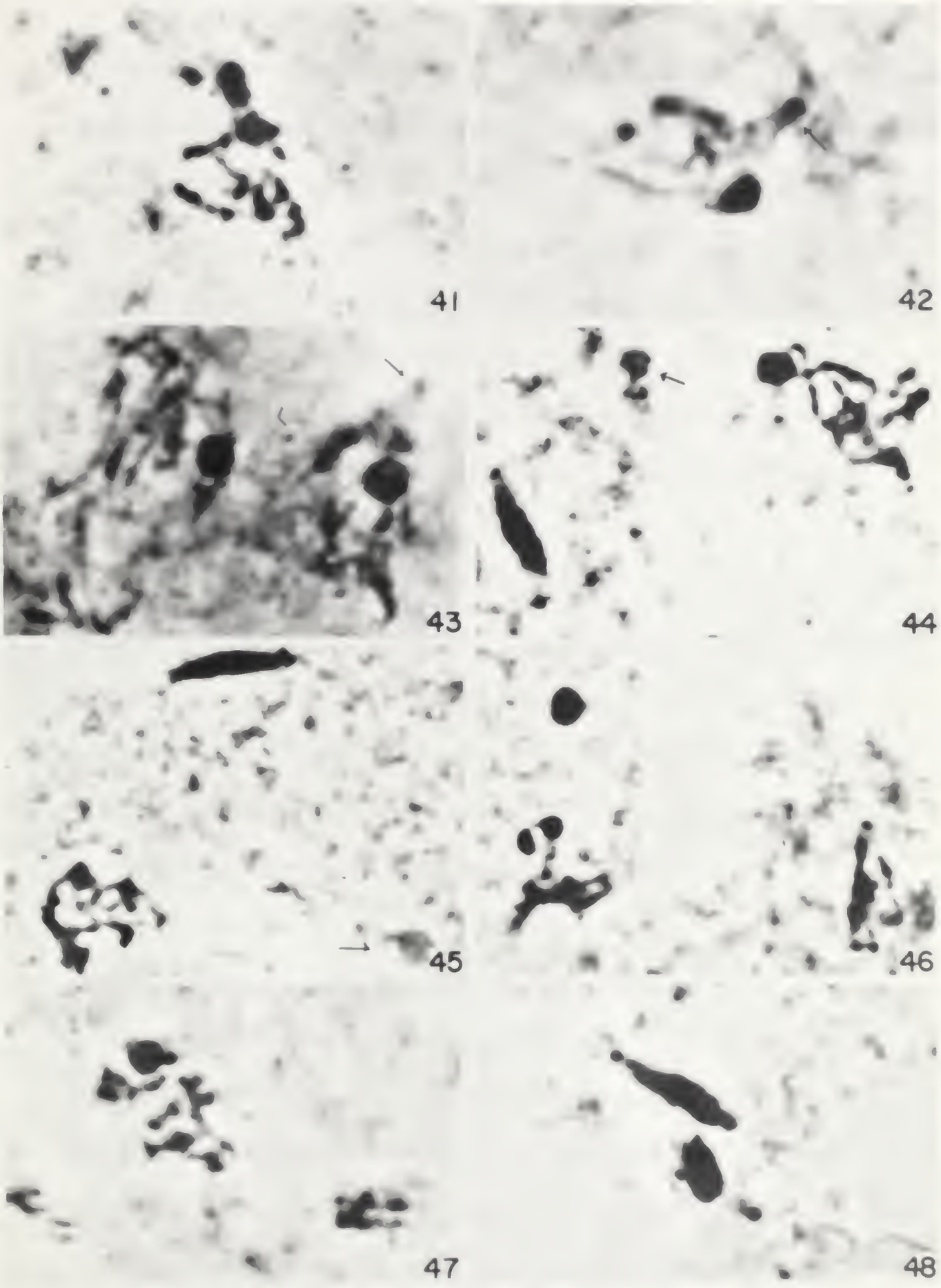


PLATE VI

These dyads do not form loops as do bivalents at diakinesis. Nevertheless, the constriction at the centromere position may be seen in some chromosomes. Metaphase II and anaphase II proceed in much the same manner as do the corresponding phases of the first division (Figs. 44-46, 48-49). Here the division of the two nuclei may be synchronous (Figs. 48, 72), or it may not (Figs. 44, 45, 47, 49). The nucleolus is released into, and disintegrates in the cytoplasm (Figs. 44-46). That the detached nucleolus is less stainable suggests that it breaks down.

The nuclei pass through telophase II to form the tetrads (Figs 50, 75) in the usual fashion. At this stage, chromosomes uncoil to the maximum. Chromatin reticulum is formed at the peripheral region of the nucleus. The nuclear membrane is again formed, and the nuclei enter into interphase II (Fig. 50). There are no cross walls between these nuclei in the basidium.

2. Mitosis

Spore Delimitation and the Third Nuclear Division

After tetrad formation, the basidium produces four outgrowths, the sterigmata. The distal end of each sterigma swells and a nucleus migrates into each protuberance (Fig. 76). The third division (post-meiotic mitosis) takes place in the spores, with the result that spores are usually binucleate (Figs. 51, 52, 77).

PLATE VII -- Anaphase II through the third nuclear division and karyotype (Cyathus stercoreus)

Figs. 49-50. (magnification 3200 X) Anaphase II to Tetrads.

Fig. 49. Anaphase II (below), late telophase II (above).

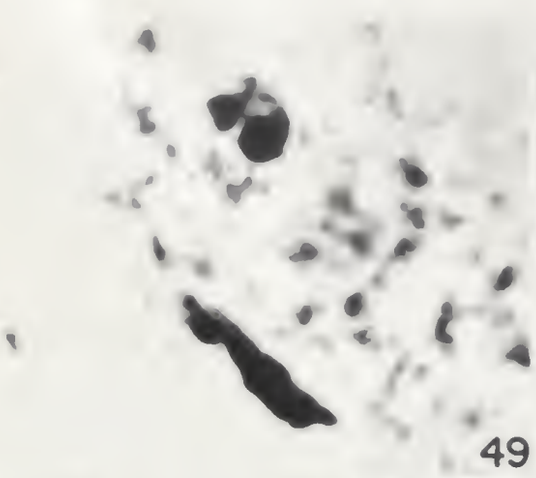
Fig. 50. Tetrads, note chromosomes are forming a reticulum.

Figs. 51-52. (Magnification 3600 X) The third nuclear division:

Fig. 51. Third nuclear division in a basidiospore, forming binucleate spores. Note chromosomes are forming a reticulum.

Fig. 52. Two nuclei squeezed out of a matured basidiospore. Note the structure of the resting nucleus.

Fig. 53. Karyotypes (cut out from the photograph shown in Fig. 19) 4000 X, showing 12 bivalent chromosomes. Note chromosomes falling into two sub-sets of 6 each; within each sub-set, chromosome patterns fall in pairs. Compared with the idiograms shown in Fig. 87. Note the bending of the long arm of chromosomes 1 and 2, as well as that of the long arm of chromosomes 7 and 8.



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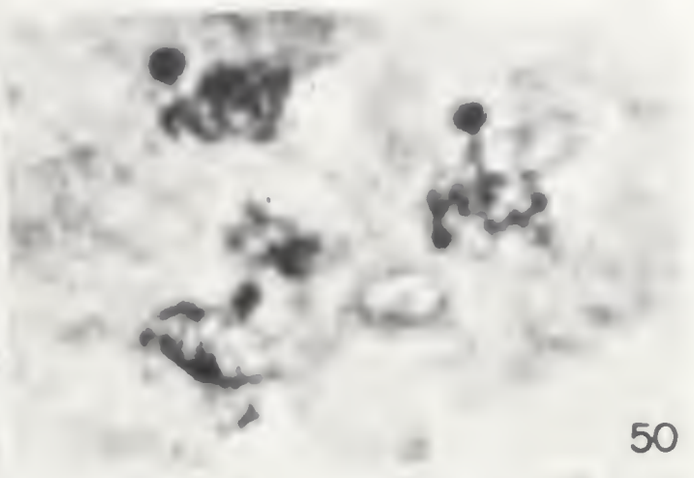

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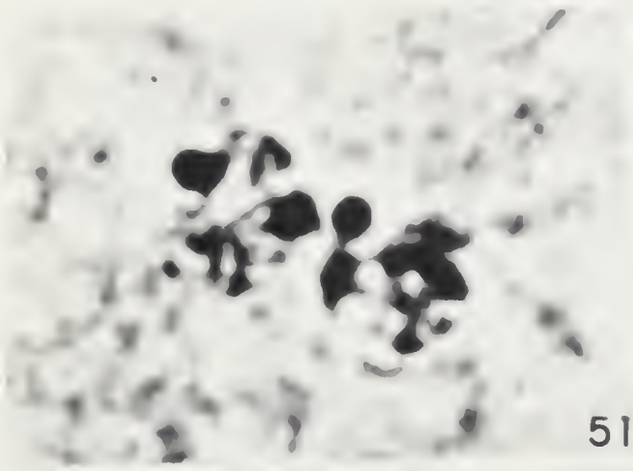

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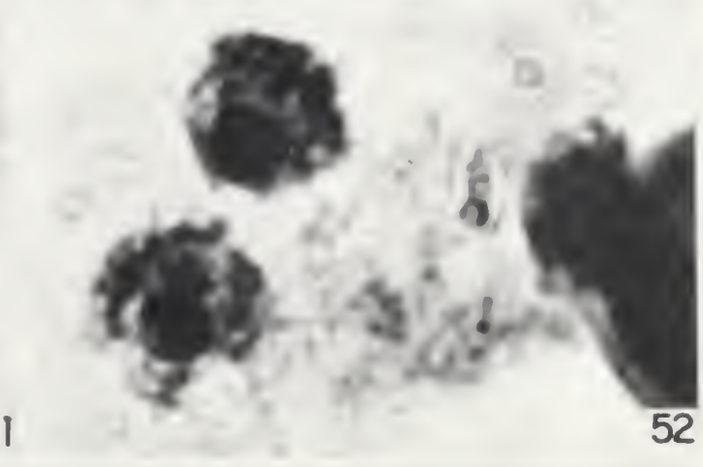

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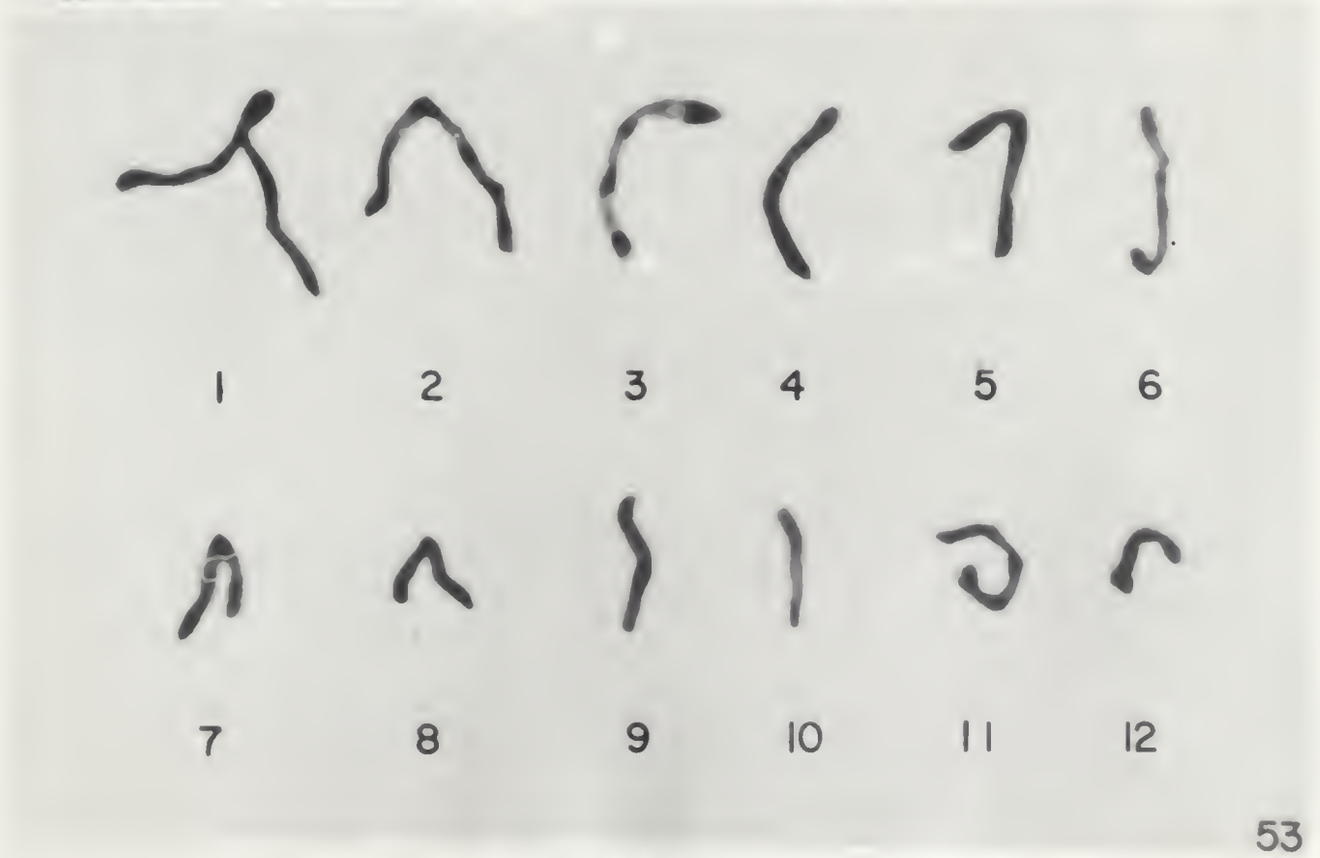


1 2 3 4 5 6

7 8 9 10 11 12

53

PLATE VII

PLATE VIII -- Presynaptic nuclei and Synapsis (Cyathus olla)

Figs. 54-57. Presynaptic nuclei:

Fig. 54. Two nuclei in the neck of an ultimate cell of a sporogenous hypha, nucleoli fully developed.

Fig. 55. Two nuclei (in the penultimate cell) undergoing telophase uncoiling of the previous presynaptic mitosis, nucleoli not yet fully organized. Note, the young basidium developing from the clamp connection.

Fig. 56. Two nuclei approaching each other in the neck of a developing basidium.

Fig. 57. Two nuclei approach each other, note thread-like chromosomes.

Fig. 58. Synapsis, showing thread-like chromosomes; note the satellite of the nucleolus chromosome. Two nucleoli are far apart.

Fig. 59. Post-synapsis, note the fused nucleolus connected to a chromosome. (That the nucleolus remains in the penultimate cell suggests that synapsis may have taken place in the penultimate cell before the nuclei moved into the basidium.)

Fig. 60. Post-synapsis (b); Presynapsis (a), two nuclei in the neck of the penultimate cell of the sporogenous hypha, note, basidium developing from the clamp connection (arrow pointed).

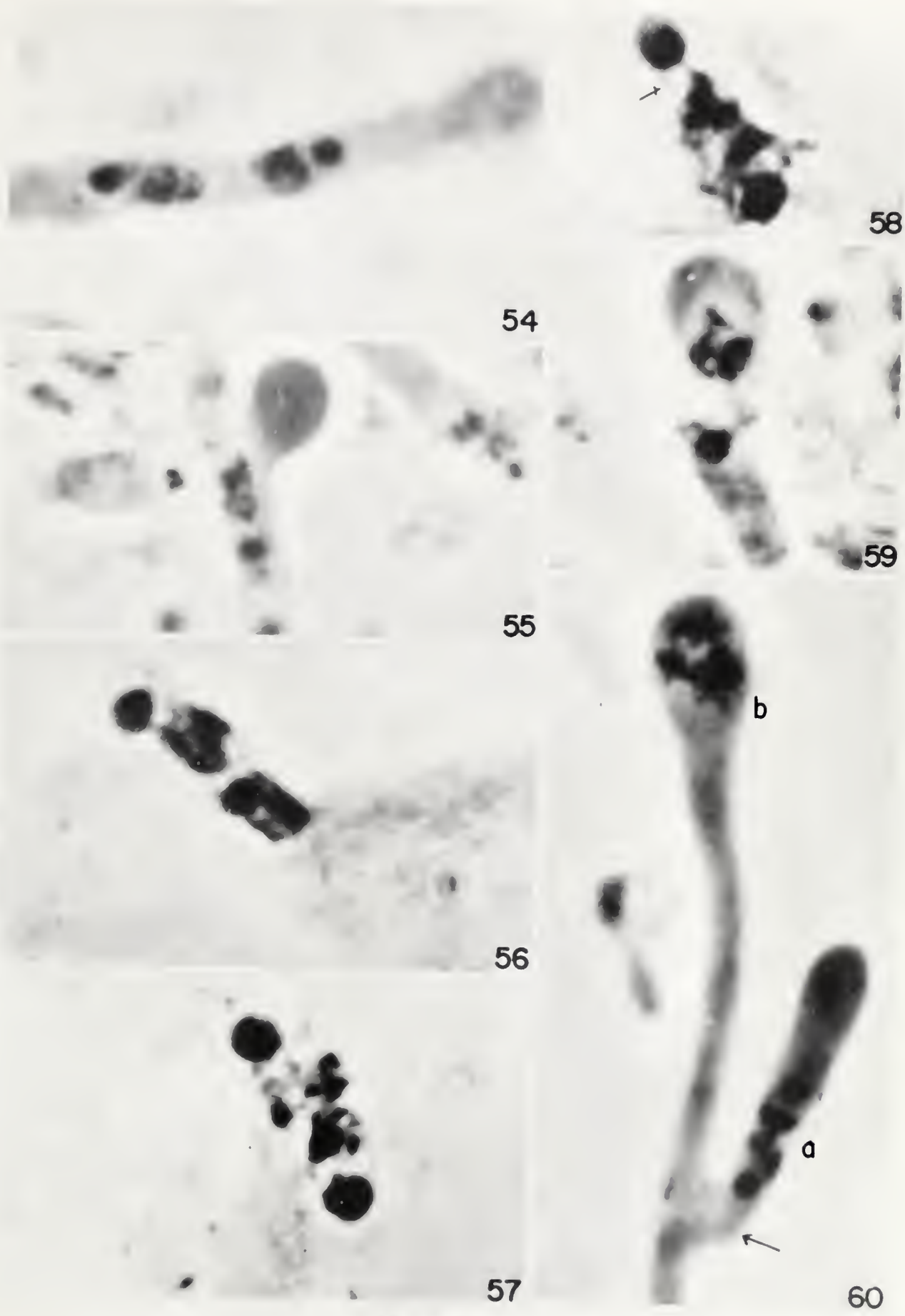


PLATE VIII

PLATE IX -- Progressive stages of Pachytene to early Diplotene (Cyathus olla)

Fig. 61. Post-synapsis, nucleoli have fused, the zygote nucleus in the main body of the basidium.

Fig. 62. Early pachytene, showing thread-like chromosomes.

Fig. 63. Pachytene, chromosomes shorter and thicker.

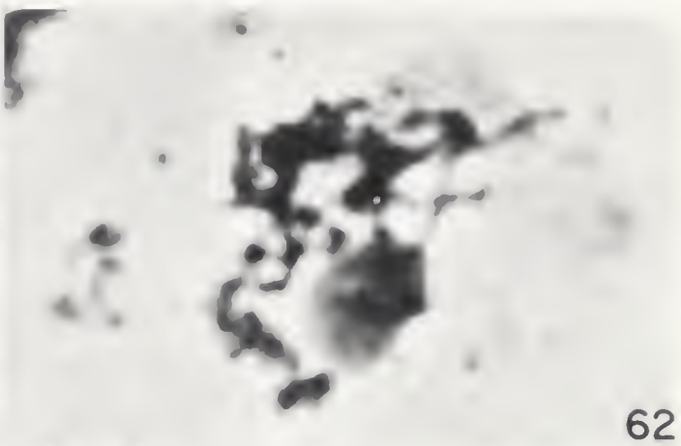
Figs. 64 and 65. Pachytene, showing the bivalent nature of chromosomes.

Fig. 66. Pachytene, showing aberrant pairing of chromosomes (arrow pointed).

Fig. 67. Early diplotene, showing loops and a few relational coilings of the homologous chromosomes. Chromosome complement not complete.

Fig. 68. Early diplotene, showing chiasma (arrow pointed).

61



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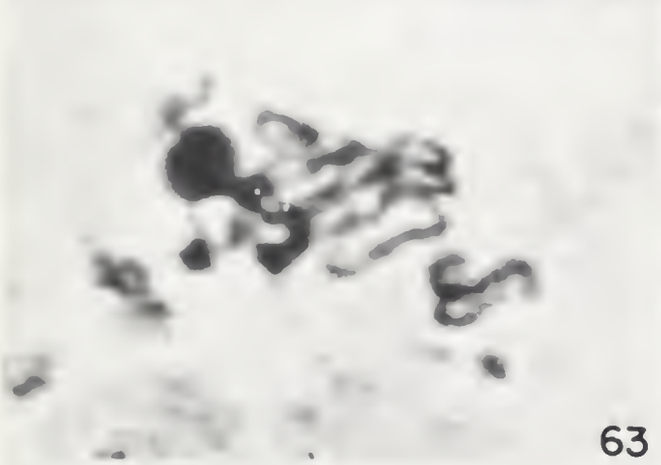

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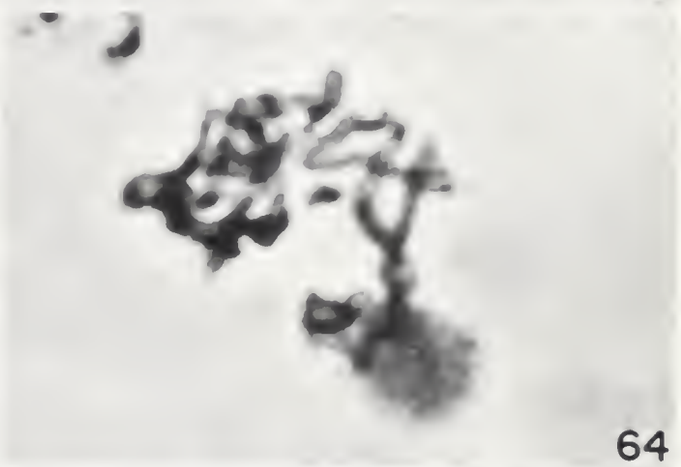

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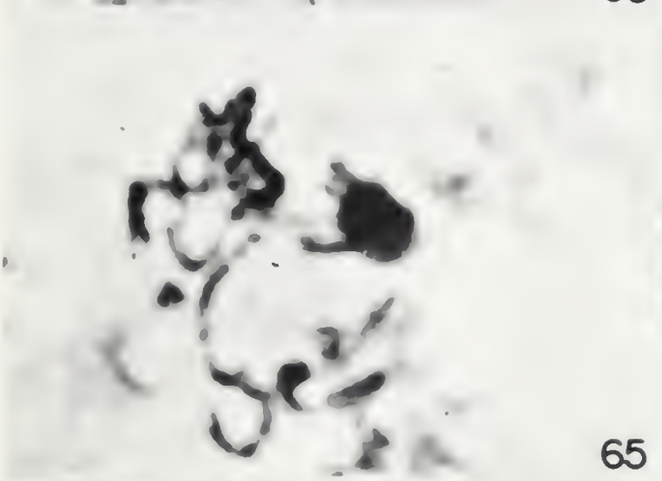

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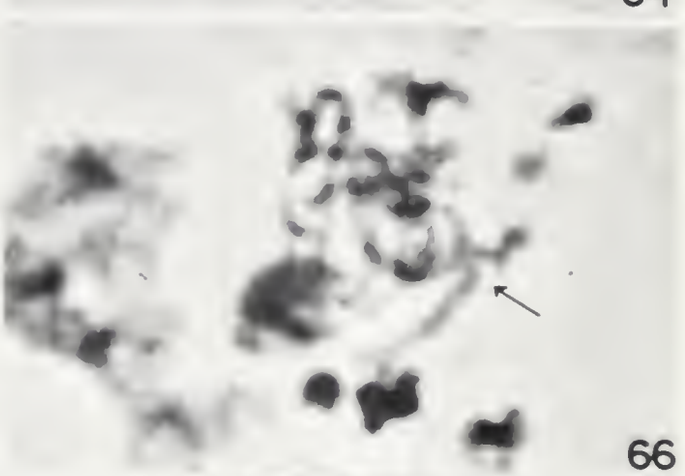

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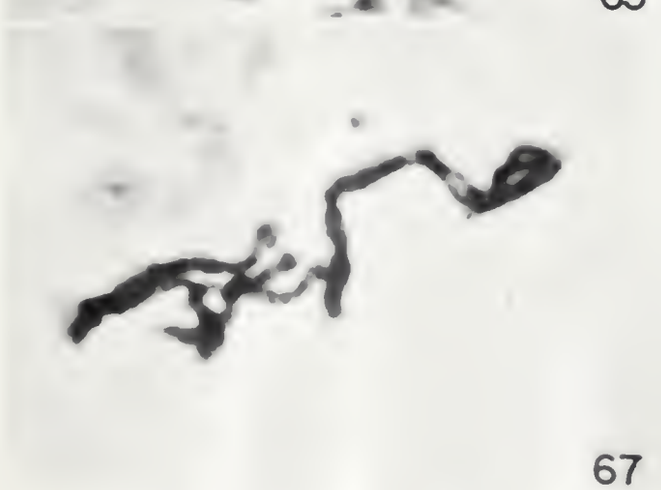

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PLATE IX

PLATE X -- Late diplotene through Binucleate spores
(Cyathus olla)

Fig. 69. Late diplotene.

Fig. 70. Late diplotene, showing aberrant pairings (arrow pointed).

Fig. 71. Early diakinesis.

Fig. 72. Diakinesis, showing 12 chromosomes. Chromosomes are more uniform in size and shape compared with those of C. stercoreus.

Fig. 73. Metaphase I, the long axis of the spindle is at right angles to the long axis of the basidium

Fig. 74. Metaphase II.

Fig. 75. Tetrads.

Fig. 76. Four spores formed from the basidium.

Fig. 77. Mature spores, binucleate.

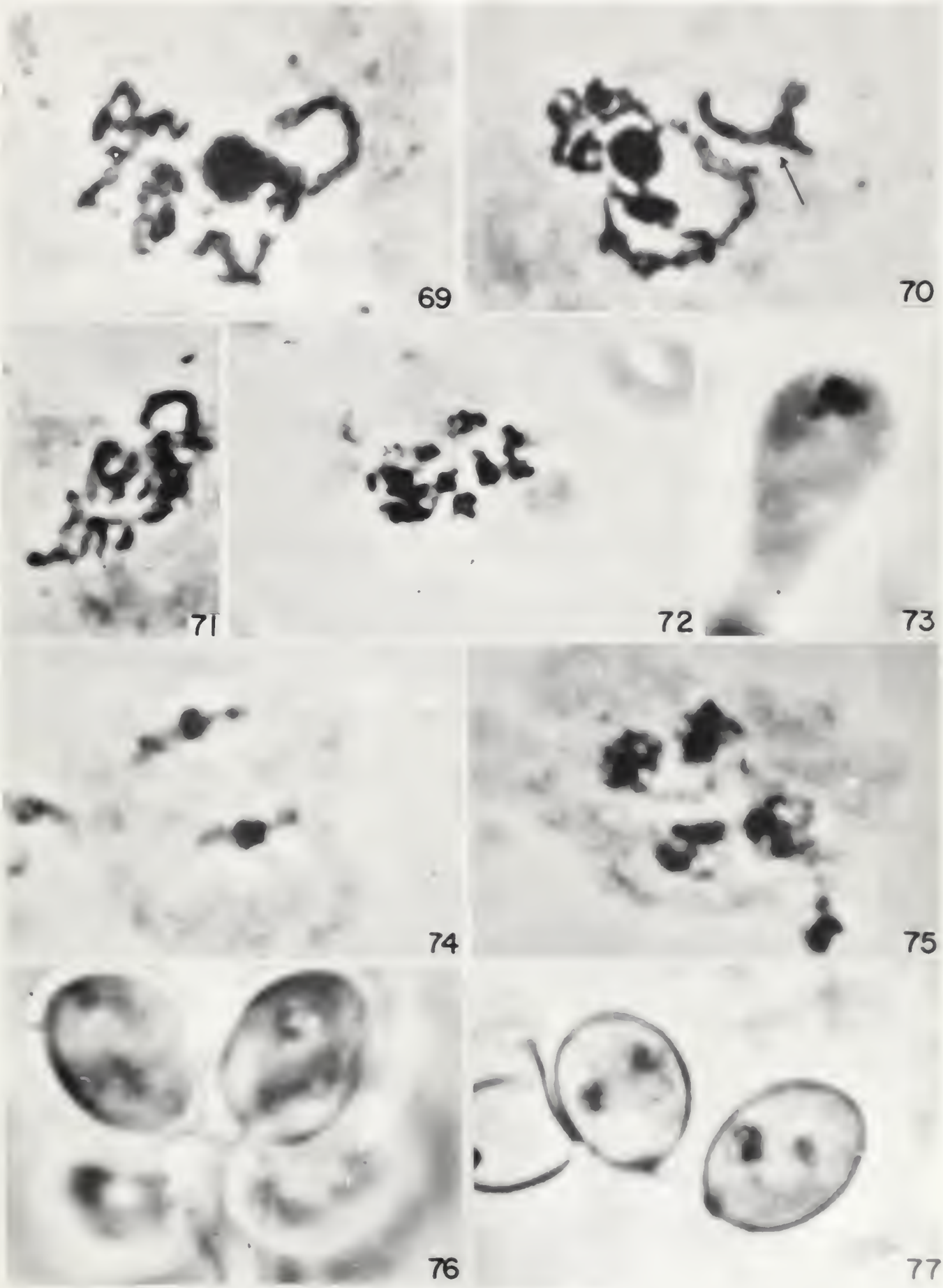


PLATE X

The structure of a "resting" nucleus is demonstrated clearly in Fig. 52 which shows two nuclei squeezed out of a spore. A "resting" nucleus consists of the nuclear membrane, the chromatin reticulum which is formed from the entanglement of chromonemata, a nucleolus, and the nucleoplasm. The nuclear membrane is not demonstrated by the technique used, but its presence is implied by the fact that the nucleus retains its integrity even after it is pressed out of the spore. Within the membrane is the chromatin reticulum, which occupies the peripheral region of the nucleus. On one side of the nucleus is a nucleolus, which is attached to a particular chromosome (chromosome 12). That the nucleolus is so attached may account for the fact that it is always on one side of the nucleus near the membrane. Inside the chromatin reticulum is the nucleoplasm that makes up the central sphere.

Mitosis in the Vegetative Mycelium

Mitosis is essentially the same in the monokaryotic and in the dikaryotic mycelium except that, in the latter, two nuclei undergo conjugate division. To avoid unnecessary repetition, only the scheme of mitosis in the dikaryon will be described.

At the onset of the nuclear division, the nucleus expands somewhat while the nuclear membrane is still present (Fig. 78). In a dikaryon, the apical nucleus proceeds to divide slightly earlier than the proximal one. However, exceptions have also been seen (Fig. 84).

PLATE XI -- Mitosis in the Dikaryon Mycelium
(C. stercoreus)

Fig. 78. Resting nuclei, note the nucleus to the left is expanding faster than the other one.

Fig. 79. Prophase, showing chromosomes of two nuclei, free in the cytoplasm, entangling one another.

Fig. 80. Prophase.

Fig. 81. Late prophase, chromosomes are shorter. Arrow points to the centriole.

Fig. 82. Prometaphase (left); anaphase (right), the nucleolus freed to the cytoplasm.

Fig. 83. Metaphase (right), chromosomes align at the equatorial plate, the centriole is seen (arrow pointed); anaphase (left), one of the poles enters the clamp connection.

Fig. 84. Early anaphase (right) showing centriole (arrow pointed), note the nucleolus, free in the cytoplasm; anaphase (left), showing the lagging chromosomes along the spindle. Arrow points to the centriole in the clamp connection.

Fig. 85. Late anaphase, daughter chromosomes have reached the poles, chromosome arms not yet contracted. At "12 o'clock" position is the clamp connection in which is a daughter nucleus.

Fig. 86. Telophase, the nucleus to the right is fully uncoiled (in ultimate cell); the nucleus to the left is uncoiling; a daughter nucleus is still in the clamp connection (above).

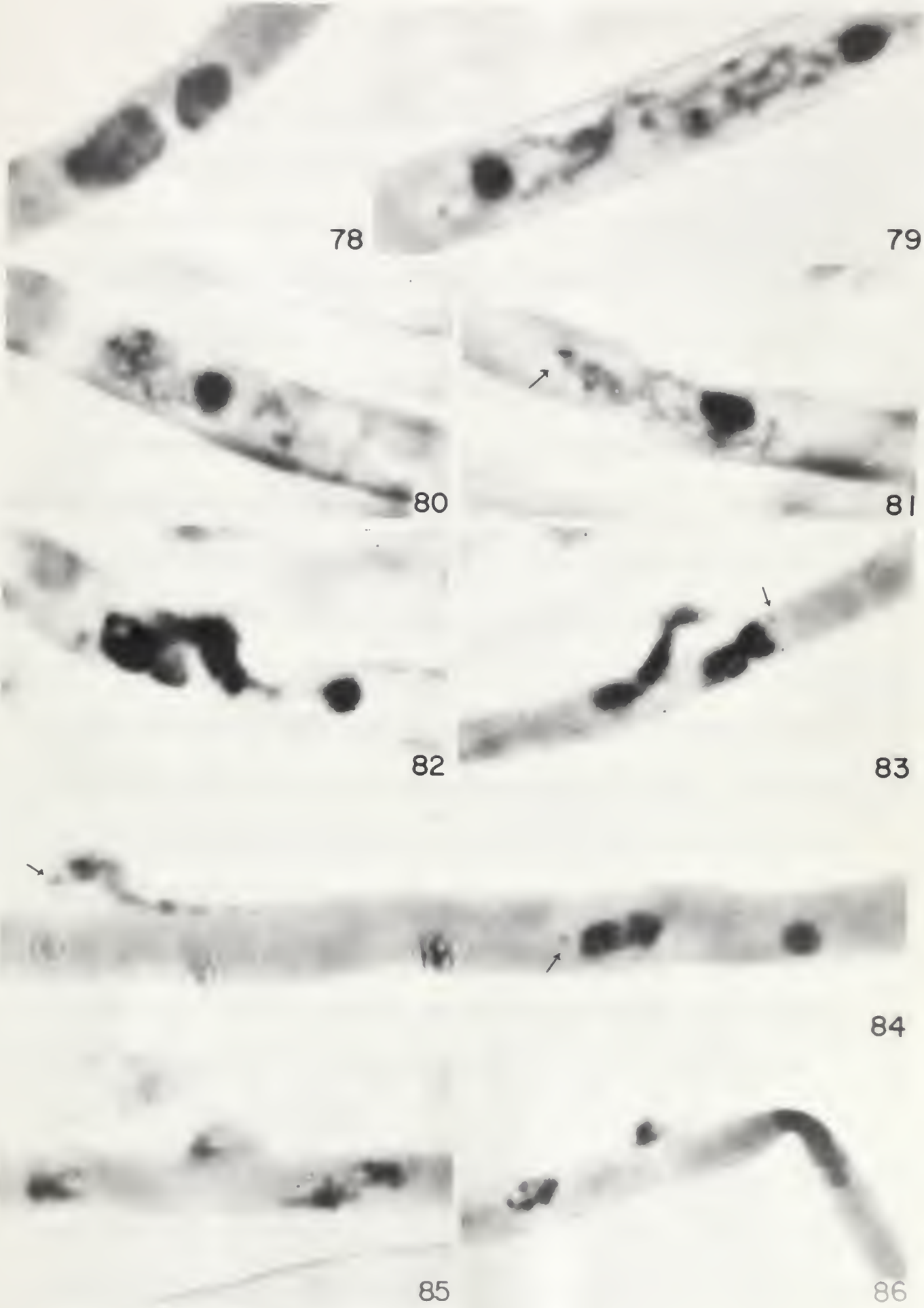


PLATE XI

Prophase: At early prophase, the nuclear membrane disappears allowing chromonemata to become free in the cytoplasm (Figs. 79-81). The chromonemata (of both nuclei) often become entangled with one another, while the two nucleoli stay far apart (Fig. 79). An interesting organelle, which may be the centriole, is always found associated with the chromonemata toward the inner side of two compatible nuclei (Fig. 81). This organelle seems to draw the chromosomes of the nucleus towards it (Fig. 81). As chromonemata coil and contract (Fig. 82), the two nuclei enter metaphase one after the other. Meanwhile, a clamp connection is developed.

Metaphase: Chromosomes, now very short, align themselves around the spindle with each centromere on the equatorial plate in a manner typical of mitosis in general (Fig. 83 right). The distribution of chromosomes (haploid set) on either side of the plate is at random. The nucleolus is now freed into the cytoplasm and disintegrates (Figs. 82, 84). The two nuclei are not exactly synchronous in behavior. The one nearest the clamp connection (mostly the apical one) always divides first (Figs. 82-84). Centrioles are discernible (Figs. 83-84).

Anaphase: Chromatids of the first nucleus split apart and move poleward, again not synchronously (Figs. 83 left, 84) as already shown for meiosis. One of the two spindle ends goes directly into

the clamp, while the other remains in the cell. Following the division of the first nucleus, the second nucleus undergoes the same process. However, the poleward movement is such that each daughter nucleus passes to one side of the clamp connection (Fig. 85).

Telophase: Chromosomes of the four nuclei begin to uncoil (Fig. 85), and a nucleolus is again formed by each nucleus. Meanwhile, a cross wall is formed in the clamp connection. This encloses the two nuclei, one of each mating type, in the ultimate cell. The clamp connection joins with the penultimate cell and releases the daughter nucleus into it, thus completing the mitotic process. During the telophase uncoiling, the nucleus in the penultimate cell undergoes uncoiling earlier than the one released from the clamp connection (Fig. 86).

3. Centriole and Spindle Mechanism

Since the centriole and spindle mechanism play in important role in the chromosome cycle, they deserve special attention. Unlike that of the Ascomycetes (15, 23, 39), the centriole of this member of the Basidiomycetes is spherical or ovoid (Figs. 34-37, 44, 46, 48, 74, 81, 83-84). Except in the mycelium and in interphase, a centriole has not been discerned in meiotic prophases. The behavior of this organelle has not been studied in detail; however,

the existence of two centrioles at nuclear fusion (Fig. 6) suggests that a fusion of these organelles may take place. On the other hand, the existence of two centrioles at metaphase implies that the fused centriole must have divided somewhere in prophase as was seen in Gelasinospora (26, see Lu's Fig. 11).

In Cyathus, there are two kinds of spindles; a continuous spindle that is formed between two centrioles (the one around which chromosomes are orientated at metaphase), and the chromosomal fibres (the ones that connect the centromeres of the chromosomes to the centrioles). Fig. 38 suggests that the latter are definite in number. The chromosomal fibres are more readily stainable than is the continuous spindle (Figs. 34, 36, 38). That there are two kinds of spindles is well shown in Fig. 34 where two entities are discernible.

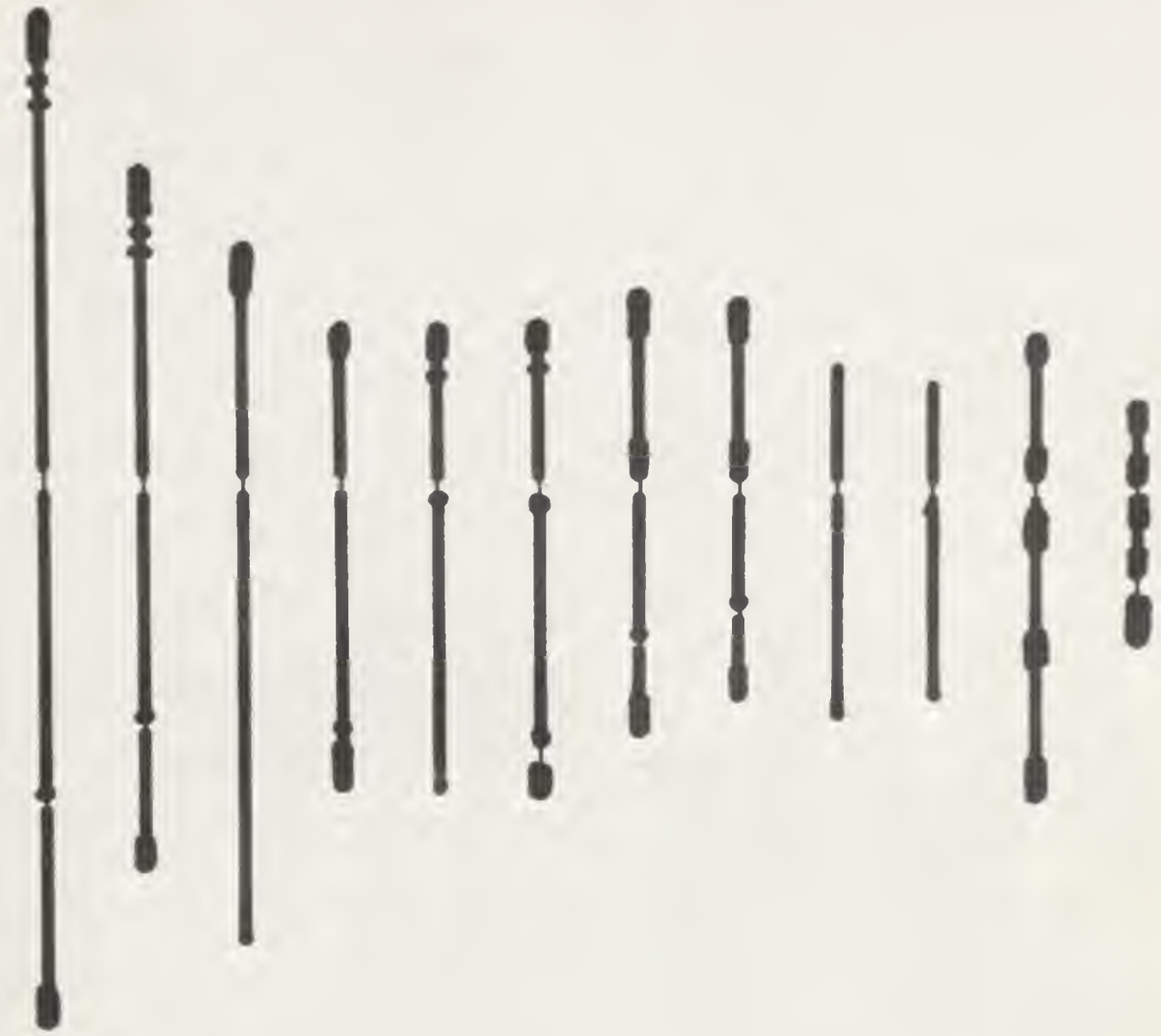
CHROMOSOME MORPHOLOGY

The morphology of individual chromosomes was delineated by: (a) the comparative length of chromosomes at pachytene stage; (b) the position(s) of the heterochromatin knob(s) along the chromosome arms; and (c) the position of the centromere. In naming chromosomes, McClintock's (29) numerical system was followed. The position of the centromere of a chromosome was determined by the chromosome

shape (such as V's or J's which were seen consistently in many pachytene or diplotene configurations). Diakinesis configurations also helped to verify the identification. The karyotypes as cut out from the photographs of Fig. 19 and rearranged are shown in Fig. 53. The idiograms are shown in Fig. 87.

Of the 12 chromosomes, there are two putative sets of 6: one has large chromosomes, the other has small ones (Figs. 17-20, 23-24, 27-31). Within these two sets, chromosome patterns fall into pairs; that is, chromosomes 1 and 2 are of the same type, and so on (Fig. 53). However, this does not necessarily mean that chromosomes of the same type are exactly the same; some differences are discernible at least in some pairs.

Chromosomes 1 and 2: Except that chromosome 1 is slightly longer than chromosome 2, both chromosomes have a near-median centromere (Figs. 17-20, 26-28, 31). Chromosome 1 has, on the tip of its short arm, a large heterochromatin knob which perhaps consists of three knobs in a sequence (Figs. 17, 19, 20). On its long arm, apart from the terminal knob (telomere), a small knob following a secondary constriction is situated about two fifths of the way along the arm towards the tip (Figs. 13, 14, 19). On chromosome 2, the telomere of the short arm is slightly larger than that of the



1	2	3	4	5	6	7	8	9	10	11	12
L/S											
1·16	1·24	2·0	2·0	2·0	2·0	1·28	1·27	2·0	2·07	2·0	2·0

Fig. 87

PLATE XII

short arm of chromosome 1 (Figs. 19-20). Whether or not chromosome 2 has also a small knob (apart from the telomere) on its long arm as does chromosome 1 cannot be decided with certainty; however, an examination of Fig. 53 where the long arm of chromosome 2 bends as does that of chromosome 1 suggests that chromosome 2 may also have the small knob following the secondary constriction. At late pachytene, chromosome 1 measures 6 μ for the short arm and 7 μ for the long arm. Corresponding measurements for chromosome 2 are 4 μ and 5 μ respectively.

Chromosomes 3 and 4: Chromosome 3 has, on its long arm, a very distinct loop which might be attributed to a paracentric inversion on one of the homologues (Figs 12, 14, 17, 19, 21). Chromosome 4 is relatively plain; it has but two discernible terminal knobs in succession on its long arm (Figs. 17, 19, 20, 23). These two knobs often merge into one in later stages (Fig. 23). The centromere of chromosome 3 has not been determined with certainty. It may also have a subterminal centromere, as does chromosome 4 (Figs. 19, 20, 23). If so, then chromosome 3 measures 3.0 μ for the short arm, and 6.0 μ for the long arm; chromosome 4 measures 4.0 μ and 4.0 μ respectively.

Chromosomes 5 and 6: Chromosome 5 is rather plain. It differs from chromosome 4 in not having 2 conspicuous terminal and

subterminal knobs on its long arm (Fig. 17). It also differs from chromosome 6 in that the latter is characterized by having three conspicuous heterochromatin knobs on its long arm: one is immediately next to the centromere, one is terminal and one is subterminal (Figs. 17, 19, 23). The terminal one may be a satellite (Figs. 17, 23, 30). Both chromosomes have a subterminal centromere (Figs. 19, 21), and both chromosomes measure 2 μ for the short arm, and 4 μ for the long arm.

Chromosomes 7 and 8: Except for being much smaller in size, these resemble chromosomes 1 and 2 in having a near median centromere (Figs. 17, 19, 23). At pachytene, chromosome 7 measures 2.5 μ for the short arm, and 3.2 μ for the long arm, whereas chromosome 8 measures 2.2 μ and 2.8 μ respectively.

Chromosomes 9 and 10: For these, the centromere is subterminal (Fig. 23). Structurally they are plain (Figs. 17, 19). At pachytene, chromosome 9 measures 1.5 μ for the short arm, and 3.0 μ for the long arm; whereas chromosome 10 measures 1.3 μ and 2.7 μ respectively. Because of the smallness, these two chromosomes often appear rod-shaped (Figs. 17, 19); however, they may appear J-shaped, as seen in Fig. 23.

Chromosomes 11 and 12: These are quite different in structure from the rest. They have five sizable heterochromatin knobs

throughout their length. These knobs with constrictions between them eventually make the chromosomes become so curved that they almost form a ring (Figs. 15, 17, 19, 22). This characteristic makes unequivocal identification of these two chromosomes possible. The fact that the chromosomes curve inward has made the determination of the centromere position difficult. It may be assumed that these two chromosomes are also subterminal, judging from Figs. 17, 19, 22 and 23. If so, chromosome 11 measures probably 2 μ for the short arm and 4 μ for the long arm; chromosome 12 measures 1 μ and 2 μ respectively. Chromosome 11 by measurement does not seem to be the second smallest, but it may be judged so because of its curved form. On this account, it would seem reasonable to pair it with chromosome 12. Chromosome 12 is the nucleolar chromosome; it is always connected with the nucleolus unless too heavily squashed. Its terminal knob is a sizable satellite (Fig. 19) which makes it differ from chromosome 11.

From the analysis of chromosome morphology, one is convinced that the whole chromosome complement may be recognized as comprising two sub-sets in which the chromosome patterns fall into pairs. The evidence for this is presented in Fig. 53.

THE POLYPLOIDY OF CYATHUS STERCOREUS

Whether or not the apparent division of 12 chromosomes into two sub-sets has any evolutionary significance, is not yet sufficiently well established to permit constructive comment. However, that the chromosomes do fall into pairs with regard to the size and the centromere position, suggests strongly that C. stercoreus may be an autotetraploid species. Evidence that supports this suggestion is as follows:

Karyotype: It was found that, of 12 chromosomes, karyotypes fall into closely-resembling, if not identical, pairs. This is to say that chromosome 1 resembles chromosome 2, chromosome 3 resembles chromosome 4, and so on. The resemblance in karyotypes is consistent in pachytene (Figs. 17, 19), in diplotene (Figs. 20, 23), and even moreso in diakinesis (Figs. 26-31). In order to substantiate the statement concerning polyploidy more clearly, the following mathematical consideration is applied.

Of the characters that contribute to the resemblance of karyotypes to one another, the centromere position of the chromosome is the most important one. If one assumed that there are four possible centromere positions (namely, median, near median, subterminal, and terminal) for any chromosome, the logical possibilities for random distribution of the centromere on 12 chromosomes

would be $4^{12} = 11,\ 657,\ 216$. Of these possibilities, there are only $_{12}P_4 = 12 \times 11 \times 10 \times 9 = 1188$ permutations that might bring about the result observed. Thus the probability for the chromosomes being so paired for the centromere position by chance alone would have been $1188/11,\ 657,\ 216 = 0.00011$. This is far too small a probability to imply that chance alone could explain the result satisfactorily.

Chromosome groupings: It was observed consistently that the like chromosomes tend to be grouped together or nearly so, even in heavily squashed preparations. This secondary association has been observed in pachytene (Figs. 17, 19), in diplotene (Figs. 23-25), and, very significantly, in diakinesis (Figs. 26-31). If one assumed that autotetraploidy is not involved and that chromosomes are randomly distributed, then one might ask what would be the probability by chance alone that the like chromosomes would be grouped together as observed. Under the assumption given above, one considers that all chromosomes are alike with regard to distribution. This situation then can be compared to an ordered partition with 6 cells which can be formed from a set of 12 elements. The total distinguishable ordered partitions would be

$$\binom{12 + 6 - 1}{12} = \binom{17}{12} = 6188.$$

Of these ordered partitions, only one case could bring about the result of like chromosomes being grouped together. Thus, the probability would be 1/6188 = 0.00017. Again, this is far too significant for chance alone to be the determining factor. In other words, the like chromosomes must have been derived from the same or a closely related origin in the evolutionary process.

Quadrivalent chromosome formation: According to Swanson (41), autopolyploids are characterized and identified cytologically by the presence of multivalents formed at metaphase of meiosis I. Evidence of multivalent pairings has been found consistently in many chromosome configurations (Figs. 23-25, 27-31). One of the most striking and conclusive is found in Fig. 23 where the units of the sub-set of 6 small chromosomes are completely paired in three quadrivalents. The high degree of multivalent pairing shown in Fig. 23 and other figures enhances the possibility that C. stercoreus may be an autotetraploid species. This conclusion is in agreement with Stebbins' (40) statement, that the difference between the allotetraploid and the autotetraploid, with regard to multivalent pairings, is a matter of degree.

Polyploidy is a common occurrence in the plant kingdom. We should not be surprised to find that C. stercoreus may be an autotetraploid species if the opinion of mycologists is valid that

the Gasteromycetes (which group includes Cyathus) are among the derived and more highly developed forms of basidiomycetous fungi.

DISCUSSION

The study of precocious synapsis from stained preparations does not ensure unbiased interpretation. However, until a better technique is available, it is necessary to make the best use of what stained preparations reveal. The description of synapsis presented in this paper is not entirely in agreement with that described by McClintock (29) and Singleton (39) in Neurospora crassa, and by Carr and Olive (16) in Sordaria fimicola. The difference in interpretation suggested by the present writer was based on the following evidence:

In accordance with the chromosome cycle described above, that the nuclear membrane is again formed after chromosomes have uncoiled at the end of telophase of the presynaptic mitosis may reveal the status of the presynaptic nuclei. In Fig. 1, two nuclei are shown without nuclear membrane, whereas in Figs. 2-5, the nuclear membrane was being formed or was already formed while two nuclei were approaching each other. This suggests that the fusion of nuclei in young basidia takes place at the end of the uncoiling process of the presynaptic mitosis. This view is enhanced from the evidence given

in Fig. 55 (C. olla), where the nucleolus is not fully organized as yet during the telophase uncoiling, and where a basidium is developing from the clamp connection of the penultimate cell.

Following nuclear fusion, synapsis takes place. During this period, chromosomes do not appear any shorter than before nuclear fusion; rather, chromosomes seem to elongate somewhat, as shown in Figs. 6-8 (C. stercoreus), and Fig. 58 (C. olla). The manner of the development of the ultimate and the penultimate cells in succession (as shown in Fig. 60) also implies this interpretation. This is to say, in Cyathus, there does not seem to be a coiling-uncoiling cycle during the course of synapsis as has been proposed for Ascomycetes (16, 20, 23, 29, 39). Neither does there seem to be a process in which the nucleoli disappear and reform as was suggested by Wells (48).

In Cyathus, the position of the nucleus (nuclei) in the developing basidium may serve as a good reference for judging the stage of synapsis (synapsis takes place in the neck of the basidium). At the completion of synapsis, whether the zygote nucleus proceeds immediately into subsequent stages, or whether it enters into a period of "resting" cannot be determined with certainty by the technique used. It might be possible that a metabolic period exists before the first meiotic cycle proceeds.

Because the meiotic cycle of the basidiomycete Cyathus stercoreus has been described and illustrated in considerable detail, further dicussion may seem unnecessary. However, in Coleosporium vernoniae, Olive (31) pointed out that frequently all of the chromosomes, at the end of synapsis, have become attached to the nucleolus. Olive's observation is not supported by the present studies. During the meiotic cycle, the second division is found to be not synchronous. This agrees with Whelden's (49-50) description for Tremellaceae.

A note about the time of each stage of meiosis may be of interest. As was mentioned, pachytene occupies a fairly long span of time during which chromosomes change their length gradually. It is probable that some time is required for the process of chromosome duplication. Diplotene and diakinesis are rather short in duration and pass quickly one after the other into metaphase. Metaphase and anaphase require a considerable length of time. The latter seems logical since, during these stages, there are required such processes as the polymerization of protein molecules to form spindles (7) around which the orientation of chromosomes takes place, and occurrences such as the movement of chromatids to the opposite poles. The telophase grouping of daughter chromosomes

in the polar position is the shortest in duration. It passes on immediately to the process of uncoiling during telophase. The same relative proportioning of time may be applied to mitosis, of which prophase is the longest phase. It should be noted that these comparative estimations of the length of time were made according to the frequencies of any stage as it occurred in the sample. On this account, the sampling errors are likely to be great.

For counting of chromosomes, metaphase is generally thought to be the best. It should be noted in the Basidiomycetes, since chromosomes at metaphase are small, they tend to clump as was pointed out by Colson (17) and as was illustrated in this paper (Figs. 32-36). For this reason, chromosome count at metaphase is not adequate. This may account for the fact that the chromosome number of many of the Basidiomycetes has been recorded as small (32). As a matter of fact, in the course of this study, an apparent chromosome count from 5-9 was obtained at metaphases. The actual number is however n = 12 for both C. stercoreus and C. olla.

That post meiotic mitosis follows the classical pattern is generally agreed. Illustrations of chromosome behavior during this division have been published by McClintock (29), Singleton (39),

El-Ani (20), Wells (48), Carr and Olive (16) and Knox-Davies and Dickson (24).

Mitosis in vegetative fungal hyphae, however, remains a subject of debate. Robinow (35, 36) and his group (8-14, 38, 43) hold the view that nuclear behavior in the vegetative hyphae is amitotic. To reconcile his scheme with the genetic processes (which cannot be explained satisfactorily by amitosis), Robinow (35) tentatively proposed "endomitosis" which takes place before the pinching of the nucleus into two daughter nuclei. The hypothesis of amitosis of Robinow and his school was based primarily on negative rather than positive proof. He classified fungi as "protists" as if they were in the same level as bacteria as was pointed out by Ward and Ciurysek (47). In his early work (35) where, for one reason or another, distinct chromosomes were not observed, Robinow argued: "The division of these nuclei cannot be described in terms of changing states of the chromatin. There is no distinct state of rest which changes into one of "prophase" which is followed by "metaphase". Here division can only be described in terms of changes of shape of the whole nucleus ..... We have therefore no evidence that they themselves are chromosomes".

However, in his scheme of endomitosis, Robinow admitted:

"It is a weakness of this assumption that it fails to account for the singleness of the nucleolus in a nucleus regarded as containing two segregated sets of chromosomes." But he argued further: "However, the relationship of the chromosomes to the nucleoli in these nuclei remains equally obscure whichever way one looks at it". In his latter work (37) where chromosomes were clearly demonstrated, Robinow was obliged to propose yet another scheme which appears no less confusing and illogical than the first.

Extending Robinow's concept, Bakerspigel (11) proposed a mechanism by means of which he endeavoured to explain chromosome movement during division. Bakerspigel's proposal that the elongation of the central body (the nucleolus) serves as a mechanical device which pushes two daughter nuclei farther apart, is very difficult to accept.

The affirmative evidence presented in this paper, together with those of others (26, 42, 46, 47), fail to confirm Robinow's concept of amitosis. This evidence, namely, the appearance of distinct chromosomes at all division stages, the existence of a spindle mechanism, the nucleolus attached to a particular chromosome (26, Lu's Fig. 5), casts serious doubt on Robinow's hypothesis. Moreover, the evidence of parasexuality in fungi (34) also supports the belief that the vegetative nuclei do not divide by amitosis as has been pointed out by Ward and Ciurysek (46, 47).

Another scheme of nuclear division was proposed by Dowding and Weijer (18, 19). They proposed that during division, chromosomes are interconnected to form a nuclear thread, which divides and splits longitudinally. Weijer and Dowding (45) also proposed that nuclear-thread interchange may offer an explanation for mitotic recombinations. If this hypothesis is correct there should be no crossing over within a chromosome. However, somatic crossing over within a chromosome was clearly demonstrated by Pontecorvo and Käfer (34).

With regard to the nature of double threads that appeared in vegetative hyphae of Neurospora (18, 19, 45), two possible explanations are suggested herewith. First, the expanded nuclei might be molded by the minute septal pores, when being forced through the latter in a fast streaming protoplast. Second, since the anaphase movement of chromatids is not synchronous, chromatids might spread out on the spindle and thus appear in two lines. Probably this is why a haploid set of chromosomes may apparently be resolved on each line. This latter explanation is perhaps more plausible than the first.

The belief that vegetative fungal nuclei divide by classical mitosis has been supported by Olive (31, 32), by Somers et al (42) and by Ward and Ciurysek (46, 47). The point of the latter authors

that the sudden changes from normal meiotic-mitotic to aberrant cycles and vice versa is not entirely logical is well taken. As vehicles for the genetic material, should these chromosomes have to behave according to different mechanisms at different times, regular genetic processes would not have been as found.

The study of nuclear behavior in a dikaryon has the following advantages: (a) there are two nuclei in a cell; (b) the formation of a clamp connection which marks the status of the nuclei as to predivisional or post-divisional stage; and (c) the apical nucleus undergoes division slightly earlier than the proximal one. The latter phenomenon provides excellent information pertaining to the sequence of events, because the stage of the apical nucleus is followed subsequently by the proximal one.

Since the interphase and the prophases are so clearly demonstrated with photomicrographs, there should be little doubt about them. However, at metaphase and at anaphase, the chromosome behavior, which is of decisive importance, may require special attention. At metaphase or thereabouts, two nuclei group closely where a clamp connection is to be formed. This phenomenon was also found in other basidiomycetes (49). At this time, all chromosomes align themselves around the spindle with

each centromere on the equatorial plate, the distribution of chromosomes on either side of the plate being at random. Such arrangement of chromosomes in a mycelium is demonstrated in the proximal nucleus shown in Fig. 83. Following the metaphase is the anaphase (the apical cell in Fig. 83) in which stage all chromatids split apart and by-pass their sister chromatids as they move to the opposite poles. It is because of the by-passing and of the non-synchronous chromosome movement, that anaphase configuration often appears like a chromatin cord (Figs. 82-84), which is analogous to the configuration at anaphase II (Figs. 44-45, 47, 49).

From both the genetic and cytological point of view, it is therefore concluded that vegetative nuclei of fungi divide essentially by mitosis similar to that of higher organisms. At least, the present study supports this view as far as Cyathus stercoreus is concerned.

Chromosome Aberrations

Chromosome aberrations occur in higher plants. In fungi also, instances of reciprocal translocation have been reported by McClintock (29-30) in irradiated Neurospora strains. However, spontaneous aberration has not been found to any extent. As was noted above, in C. stercoreus, chromosome 3 has a loop on its long arm. This loop may be attributed to a paracentric inversion

on one of the homologous pairs. At synapsis, the point-to-point pairing forces the chromosome to form a loop. Because this loop is small, and because the inversion inhibits crossing over somewhat (41), a chromosome bridge accompanied with an acentric fragment (which may result from a crossing over within the loop) has not been observed. Whether this inversion produces any position effect or not remains to be studied.

Another instance of chromosome aberration was also found in C. stercoreus. A double chromatid bridge without any chromosome fragments was observed occasionally at early telophase I. The nature of these bridges is not fully understood. A bridge of this type has been reported in the Speltoid mutants of hexaploid wheat arising either spontaneously or by X-irradiation (28, 44). But the double bridge like that observed in C. stercoreous was rare in hexaploid wheat. According to Mac.Key (28) and Uchikawa (44), a bridge of this kind may be attributed to the delayed terminalization of chiasmata following semi-homologous crossing over. It is conceivable that, since C. stercoreus is probably an autotetraploid species, the semi-homologous crossing over may be a plausible explanation for the bridges observed. Moreover, autotetraploidy might account for the double bridges in C. stercoreus, rare in such allopolyploidy as found in hexaploid wheat.

In C. olla, on the other hand, chromosome configurations seen at late pachytene (Fig. 66) and at diplotene (Fig. 70) suggested either a possible reciprocal translocation or an illegitimate pairing due to polyploidy. Because C. olla was not studied in any detail, speculation on this point is of little value.

Polyploidy

Polyploidy is known to a greater or lesser degree in all groups of plants. It has been found in algae, in bryophytes, and in vascular plants, especially angiosperms. In fungi, however, polyploidy is apparently rare or lacking (Stebbins 40). The apparent scarcity of polyploidy in fungi may be due to the lack of reliable evidence regarding chromosome numbers, especially for the Basidiomycetes.

Polyploidy is the best understood of the mechanisms which contribute to species formation (41). It would not be surprising to find that C. stercoreus is a tetraploid species since the Gasteromycetes (to which C. stercoreus belongs) are considered by many to be the highest of fungi with regard to evolutionary trend.

The classification of C. stercoreus as an autotetraploid species is based on the following kinds of evidence: (a) karyotypes, (b) grouping of the like chromosomes at pachytene through diakinesis, and (c) frequent occurrence of quadrivalent pairings.

Karyotype analysis revealed that twelve chromosomes of C. stercoreus could be partitioned into two equal sub-sets (6 each): one group has large chromosomes, and the other has small ones. Whether or not this bipartition of karyotypes has any evolutionary significance is conjectural. It might suggest that a remote allotetraploidy had been involved. From this allotetraploid species, again autotetraploidy might have taken place. This would mean that C. stercoreus might have been an autoallopolyploid (the octaploid) species with the basic chromosome number n = 3.

It should be noted that the discussion given above is admittedly speculative and should not be taken too literally. However, such speculation suggests that it would be of interest to know the relationships between the chromosome numbers of the most primitive and those of the more advanced forms of the Basidiomycetes.

Another question that arises in connection with the ploidy is the possible course of evolution of sexuality. In fungi, it has been suggested that the Basidiomycetes evolved from the Ascomycetes (1, 15). This view is enhanced by the fact that the basidium is developed from the clamp connection (Fig. 60) which is analogous to the ascus formation. Since the majority of the Ascomycetes are known to be bipolar for sexuality, whereas the majority of the Basidiomycetes are tetrapolar (1), it may be that sexual tetra-

polarity evolved from bipolarity through the process of tetraploidy. According to Alexopoulos (1), of all species of heterothallic Basidiomycetes, 37% are bipolar, and 63% are tetrapolar. It would be interesting to look into the correlation between the polarity of sexuality and the ploidy of the chromosome complements.

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